The Structure and Function of MUSCLE

Volume I

Errata

Page 92, lines 12 and 23
Page 97, line 2
Page 105, line 7
For "page 000" read "Volume III, Chapter IX"

Volume I: Structure

Volume II: Biochemistry and Physiology

Volume III: Pharmacology and Disease

The Structure and Function of MUSCLE

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Volume I

STRUCTURE



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PREFACE

Muscle is unique among tissues in demonstrating to the eye even of the lay person the convertibility of chemical into kinetic energy.

The precise manner in which this is done is a problem, the solution of which has been pursued for many years by workers in many different disciplines; yet only in the last 15 or 20 years have the critical findings been obtained which have enabled us to build up some sort of general picture of the way in which this transformation of energy may take place. In some cases the studies which produced such rich results were carried out directly on muscle tissue. In others, collateral studies on other tissues were shown to have direct application to the study of muscular contraction.

Prior to 1930 our knowledge of muscle was largely restricted to the macroscopical appearance and distribution of various muscles in different animals, to their microscopical structure, to the classic studies of the electro- and other physiologists and to some basic chemical and biochemical properties. Some of the latter studies go back a number of years and might perhaps be considered to have started with the classic researches of Fletcher and Hopkins in 1907, who demonstrated the accumulation of lactic acid in contracting frog muscle. This led very shortly afterward to the demonstration by Meyerhof that the lactic acid so formed is derived from glycogen under anaerobic conditions. The lactic acid formed is quantitatively related to the glycogen hydrolyzed. However, it took until nearly 1930 before it was established that the energy required for the contraction of a muscle was derived from the transformation of glycogen to lactic acid.

This was followed by the isolation of creatine phosphate and its establishment as an energy source for contraction. The isolation of ADP and ATP and their relation with creatine phosphate as expressed in the Lohmann reaction, were studies carried out in the thirties. What might be described as a spectacular claim was made by Engelhart and Lubimova, who in the 1940's said that the myosin of the muscle fiber had ATPase activity. The identification of actin and relationship of actin and myosin to muscular contraction, the advent of the electron microscope and its application with other physical techniques to the study of the general morphology and ultrastructure of the muscle fibers were events in the 1940's which greatly developed our knowledge of this complex and most mobile of tissues.

In the 1950's the technique of differential centrifugation extended the knowledge obtained during previous years of observation by muscle cytologists and electron microscopists to show the differential localization of metabolic activity in the muscle fiber. The Krebs cycle and the rest of the complex of aerobic metabolism was shown to be present in the sarcosomes—the muscle mitochondria.

This is only a minute fraction of the story of muscle in the last 50 years. Many types of discipline have contributed to it. The secret of the muscle fiber has been probed by biochemists, physiologists, histologists and cytologists, electron microscopists and biophysicists, pathologists, and clinicians. Pharmacologists have insulted skeletal, heart, and smooth muscle with a variety of drugs, *in vitro*, *in vivo*, and *in extenso*; nutritionists have peered at the muscle fiber after vitamin and other nutritional deficiencies; endocrinologists have eyed the metabolic processes through hormonal glasses. Even the humble histochemist has had the temerity to apply his techniques to the muscle fiber and describe results which were interesting but not as yet very illuminating—but who knows where knowledge will lead. Such a ferment of interest (a statement probably felicitously applied to muscle), in this unique tissue has produced thousands of papers and many distinguished workers, many of whom we are honored to have as authors in this compendium.

Originally we thought, the publishers and I, to have *a book* on muscle which would contain a fairly comprehensive account of various aspects of modern research. As we began to consider the subjects to be treated it became obvious that two volumes would be required. This rapidly grew to three volumes, and even so we have dealt lightly or not at all with many important aspects of muscle research. Nevertheless, we feel that we have brought together a considerable wealth of material which was hitherto available only in widely scattered publications. As with all treatises of this type, there is some overlap, and it is perhaps unnecessary to mention that to a certain extent this is desirable. It is, however, necessary to point out that most of the overlap was planned, and that which was not planned was thought to be worthwhile and was thus not deleted.

We believe that a comprehensive work of this nature will find favor with all those who work with muscle, whatever their disciplines, and that although the division of subject matter is such that various categories of workers may need only to buy the volume which is

especially apposite to their specialty, they will nevertheless feel a need to have the other volumes as well.

The Editor wishes to express his special appreciation of the willing collaboration of the international group of distinguished persons who made this treatise possible. To them and to the publishers his heartfelt thanks are due for their help, their patience, and their understanding.

Emory University, Atlanta, Georgia
October 1, 1959

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CHAPTER I

The Anatomy of Muscles and their Relation to Movement and Posture

R. D. LOCKHART

I. MUSCLE STRUCTURE

Despite the variety of form and function throughout divergent phyla, it is an interesting reflection that the motive power throughout the animal kingdom with few exceptions is muscle (Pantin, 1956).

The muscular system confers the grace of the human body in movement or at rest. The paramount virtue of muscle, its contractility, is the basis of physical culture, a fundamental principle in many aspects of treatment in orthopedic surgery, and invaluable in the examination of the nervous system, when the clinician, by testing the

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C. Design

Apart from phylogenetic inheritance, the form of a muscle is determined by its function, which, in turn, requires compromise between power, speed, and range of movement. The more one regards the formation of the body, the more one appreciates the beauty of the design—the mass of the muscle bellies placed to avoid interference with movement and tapered to tendons or blended with aponeurotic sheets where joints must be free or where muscle is unnecessary.

The striated, voluntary, or skeletal muscles, comprising about 40% of the weight of the body, are made up of bundles of fibers—each fiber embedded in fine connective tissue (the endomysium), each bundle in a sheath of perimysium, and all the bundles of the complete muscle invested by the sheath of the muscle, the epimysium.

D. Length of Muscle Fibers

Individual fibers may be patiently dissected by hedgehog spines and a little weak alkali or acid or boiling to soften the connective tissue. Fibers may be traced from the tendon of origin to the tendon of insertion and may be more than 30 cm. long in the sartorius muscle, as shown by Lockhart and Brandt (1938). These authors never found several fibers interdigitating in the length of a muscle between the tendons of origin and insertion, an arrangement which had frequently been described.

Muscle fibers never gain direct attachment to bone, as tendon always intervenes. It is the endomysium, perimysium, and epimysium which blend with the tendon; indeed, there is not enough area of bone to afford attachment for all the muscle fibers.

E. Tendons and Fiber Arrangement

Where short muscle fibers are adequate for the movement required, tendon saves the more specialized muscle tissue. Again, the use of a long tendon cord allows a muscle belly to exert its full power upon a distal point, and, further, the tendon may ply around a pulley whereby the muscle exerts a line of pull divergent from the axis of its own length. Tendons may run along or into the body of a muscle, the muscle fibers being attached to one side in the unipennate types of muscle, to both sides in the bipennate, and giving rise to multipennate forms where several tendons invade the muscle (Fig. 1).

power of the various muscle groups, reveals the state of their nerve supply as intact or defective. Again, happiness or depression, vigor or weariness are not only emotionally registered by the facial muscles but are also depicted by the muscular system in the whole poise of the individual.

A. The Importance of Studying Muscles in the Living Body

Since many of our muscles are superficial and easily seen and felt in action, no apology is made for stressing at the outset the elementary fact, often overlooked, that the beginner may learn much about the activity of muscles, independent of all textbooks and literally at first hand, merely by resisting the movements of a companion's limbs with one hand while the other hand identifies the muscles which alternately harden with the movement and soften with the reverse movement.

More than two hundred years ago, Winslow (1732) stressed the need for experiment upon the muscles in the living subject; one hundred and thirty five years later Duchenne (1867) showed the fallacies of study by scalpel upon the dead instead of by "electrisation" upon the living; more than fifty years ago Beevor (1904) again insisted that the action of muscles must be studied upon the living body. But even seven years ago, it was still necessary for the distinguished clinician F. M. R. Walshe (1951) to state in his foreword to the reprint of Beevor's "Croonian Lectures on Muscular Movements", issued by the publishers of the journal *Brain*, that Beevor's valuable studies are already to a large extent lost and that neither the clinical neurologist nor the experimental physiologist can afford this loss today. For the details of the action of individual muscles, beyond the scope of this text, the reader will find a mine of interest in the brilliant works of Duchenne and Beevor.

B. Nomenclature

Muscles have been named from a variety of their features—for example, from attachments (sternomastoid), action (supinator), direction (rectus), situation (gluteus), structure (triceps), size (magnus), and shape (trapezius). Many are designated by composite terms such as flexor digitorum profundus. It is interesting to note, in passing, that few muscles received special names until the early eighteenth century. Previously numbers were given to muscles in regional groups, by Galen and Vesalius while Leonardo da Vinci applied letters in his illustrations.

moves, thereby gaining speed at the loss of power that a longer lever would have obtained. At the elbow, for example, a wide and rapid movement of the hand results from a comparatively small muscular contraction.

F. The Motor or Neuromotor Unit

The nerve supplying a muscle contains both motor and sensory fibers, almost half of them sensory. A single motor nerve fiber, an axon, is responsible for the innervation of a definite group of muscle fibers, sending a filament to each muscle fiber of the group. In a large muscle such as gluteus maximus, there might be 200 muscle fibers in each group, but only 5 muscle fibers in the groups comprising a small muscle moving the eyeball. An impulse in a single axon would activate the smallest number of muscle fibers that can be engaged. Accordingly, an eye muscle axon stimulating only 5 muscle fibers will produce a more delicately precise action than a gluteal muscle axon stimulating 200 muscle fibers at the least. Each muscle is a company of neuromotor units, with each unit comprising a nerve cell, its axon, and its group of muscle fibers.

In moderate activity of a muscle the units work in relays providing continued action without fatigue. The more powerful the action required, the more units act at the same time. The smaller the part of the body to be moved, the smaller the muscle, the more rapid its action, and the smaller the unit. The activity of the motor unit system may be responsible to a certain extent for the tension or tone always present in a muscle even when it is at rest, although electromyography does not support this theory. Possibly the tone is due to the inherent elasticity of the muscle. All efferent nerves to voluntary muscle stimulate the muscle to contract; there are no efferent nerves to produce relaxation. Relaxation is achieved by reducing or terminating the neuromotor stimuli. Repeated exercise, as in a course of physical training, increases the thickness of the muscle fibers, which, at birth, are twice as thick as in the fourth month embryo and one-fifth of the adult thickness.

G. The Neurovascular Hilum

Vessels and nerves enter a muscle, as a rule, at a definite cleft, the neurovascular hilum. These hila have been determined for the limb muscles by Brash (1955).

So much attention has been accorded to muscle that there is apt to be neglect of the importance of tendon in its role as buffer guarding the limbs against the danger of sudden strain and speed that muscle alone would incur. Again, a stretched tendon by its elastic recoil increases the muscular action (Hill, 1951).

Although the term "origin" is usually applied to the proximal or

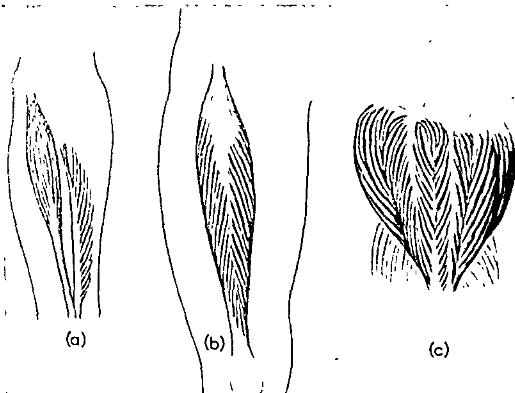


Fig. 1. Types of muscle fiber and tendon arrangement. *A*. Fusiform and unipennate: flexor carpi radialis and flexor pollicus longus. *B*. Bipennate: rectus femoris. *C*. Multipennate: deltoid

less mobile point of a muscle's attachment, the term "insertion" to the distal and more mobile attachment, the action of a muscle may be reversed. For example, in climbing up a rope the body is pulled toward the arms and the insertions of the muscles into the arms are the more fixed points. Then again, in the foot and leg the terms are unfortunate since the muscles usually act when the foot is applied to the ground.

Usually, in the limbs, a muscle is inserted just beyond the joint it

II. MUSCULAR ACTION

A. The Amount of Contraction of a Muscle Fiber

It is possible for a muscle fiber fully stretched to contract to 57% of its length (Haines, 1934), this amount generally corresponding to the degree of movement permitted at the joint between the bones involved.

B. Muscles of Short or Insufficient Action

The fibers of certain muscles, however, are so short that they do not execute the full movement possible between their attachments, as exemplified in the biceps femoris, semimembranosus, and gastrocnemius. When these muscles are fully contracted the knee is not fully bent and in further flexion of the joint these muscles, described as of short action or insufficient action, are no longer taut. Again, some muscles may be unable to relax sufficiently to allow full movement to occur at a joint in certain conditions; for example, the hamstring muscles, when the knee is extended, will not relax enough to give full flexion at the hip, a state of passive insufficiency which will be discussed subsequently under the ligamentous action of muscle.

C. Muscle Tonus or Tension; Use of the Terms Contraction, Relaxation, "Concentric" and "Excentric" Action, Active Shortening and Lengthening, Adaptive Shortening, and Lengthening

Tonus of muscles has already been discussed in the Section I, F neuromotor unit. Because of their tone, there is no slack to be taken up by a muscle before it contracts. It must be emphasized that a muscle may be described as contracted in the sense of being firm, tense, or active without implying shortening, indeed a muscle may be exerting its greatest power when fully stretched.

When the arm is raised to the horizontal, the deltoid muscle (Fig. 3) is felt firmly contracted and when it is lowered in the absence of

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Fig. 2. Activity of trapezius and sarcospinalis. The trapezius muscles are raising the arms against resistance while the two heavy ridges in the lower part of the back show the activity of the sacrospinalis muscles in maintaining the erect posture against the heavy leverage upon the arms. Reproduced from "Living Anatomy" by R. D. Lockhart (1959) (Courtesy of Faber & Faber, London; Oxford University Press, New York).



nervous system through reflexes in the spinal cord activated by stretch stimuli from the muscles and stimuli from the skin, the eyes, and vestibular structures. Posture under reflex control has been described as the basis of movement, because movement starts from and terminates in a posture; indeed, the stand at ease position, according to Hellebrandt (1938) is in reality movement upon a stationary base. Sway is inseparable from the upright stance and the altered balance

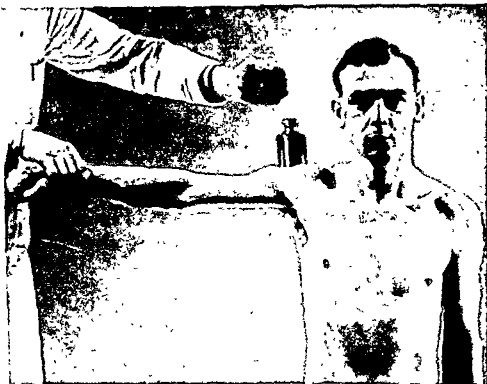


FIG. 4. Deltoid flaccid, pectorals active When, however, the arm is pulled down against resistance, the weight sinks into the suddenly flaccid deltoid and its antagonists such as the pectoral and latissimus dorsi muscles come into action. Reproduced from "Living Anatomy" by R. D. Lockhart (1959) (Courtesy of Faber & Faber, London; Oxford University Press, New York).

causes new stretch stimuli. As long as the balance is steady, in the erect posture there is very little and in most persons no detectable activity in the trunk, thigh, and anterior leg muscles but the calf muscles are active (Joseph *et al.*, 1955; Joseph and Nightingale, 1952, 1954, 1956; Floyd and Silver, 1955). The line of the center of gravity passes behind the hip joint and in front of the knee and ankle joints. While the ligaments in front of the hip and behind the knee may momentarily take the strain before muscles are employed to retrieve

resistance the muscle is still felt firmly contracted while, under tension, it controls the gravitational descent of the arm as a crane lowers a weight. In the ascending arm it is actively shortening and in the descending arm it is actively lengthening or relaxing. These actions are sometimes, in physiotherapy and physical training colleges, described respectively as "concentric" and "excentric". During the ascent of the arm the muscles which can oppose the movement must



Fig. 3 Deltoid active, pectorals flaccid. Whether the arm is being raised or lowered, the weight rides upon the firm deltoid, which not only raises the arm but also controls its gravitational descent, respectively contracting or paying out under tension as a crane lowers a weight Contrast with Fig. 4.

adaptively lengthen or relax, and during the descent they must adaptively shorten. When the descending arm encounters resistance, the deltoid (Fig. 4) becomes suddenly flaccid and adaptively lengthens while the muscles pulling down the arm become firmly contracted in active shortening

D. Muscles Maintaining Posture Against Gravity

Muscles maintaining posture against gravity, sometimes termed anti-gravity or postural muscles, are under the control of the central

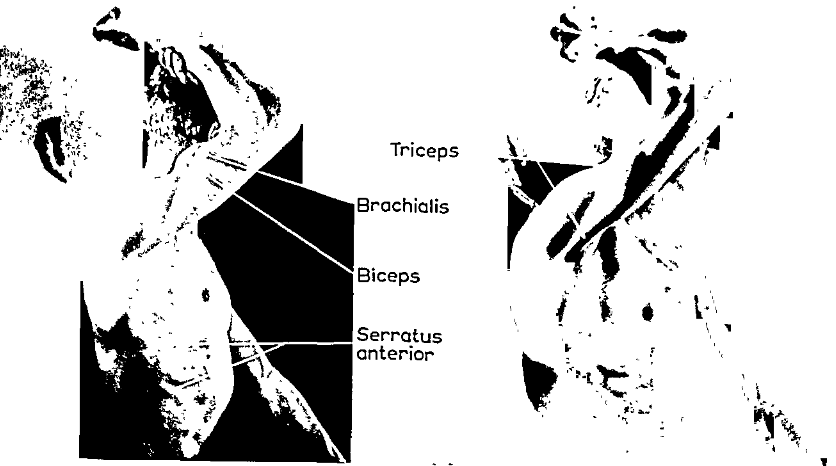


FIG. 5. Flexing elbow against resistance. Biceps and brachialis active, triceps flaccid.
FIG. 6. Extending elbow against resistance. Biceps and brachialis flaccid, triceps active. Serratus active in steadying the scapula in both figures. Reproduced from "Living Anatomy" by R. D. Lockhart (1959) (Courtesy of Faber & Faber, London; Oxford University Press, New York).

the balance, the calf muscles are always active. The delicate precision with which the trunk muscles control balance is easily appreciated by feeling the spinal muscles contract when one merely raises an arm forward (cf. Figs. 2, 10). Again, with one hand on the back and the other on the front of the trunk, the alternate contraction and relaxation of the muscles accompanies a slight to and fro movement. The activity of a muscle maintaining the erect posture is no different from that in executing a movement. Although a muscle may frequently have such a postural role, it is no different from other muscles.

The importance of muscular action in relation to gravity concerns every muscle and movement. The sternomastoid muscle is usually described as flexing the head and neck but it never does so unless it is working against resistance or gravity as in a person rising from the supine position; otherwise flexion of the head and neck is due to active lengthening of the muscles on the back of the neck. To take an example from the upper limb, stretch the arm horizontally (palm up) and then slowly bend the elbow. The biceps will be felt firm and the triceps on the back of the upper arm soft until the forearm is vertical, but the moment flexion continues across the plumb line the biceps becomes soft and the triceps firm. If both flexion and extension movements are resisted then obviously the biceps is active throughout the whole of flexion and the triceps throughout the whole of extension (Figs. 5 and 6).

Alterations in posture precede the majority of movements to secure the best attitude for the best performance. Indeed, it is a cult assiduously practiced in games.

To be caught on the wrong foot can be literally expressive. In rising from a chair the first act is to bring the feet under the center of gravity. The starting attitude of the sprinter and the natural movement of a horse in first stepping backward in order to lean its weight forward in starting to pull a heavy load illustrate the same principle.

In the standing posture, although electromyography finds only the calf muscles active, the anterior leg muscles are certainly felt firmer than in a leg which is hanging free and much firmer still if the erect person leans forwards. The anterior tibial muscle must play its part in maintaining the arch of the foot. Because of the normal action of the calf muscles plantar flexing the foot in the leg hanging free, dorsiflexion from this position, as in placing the foot upon the ground, increases the tension in the calf muscles. With the foot upon the

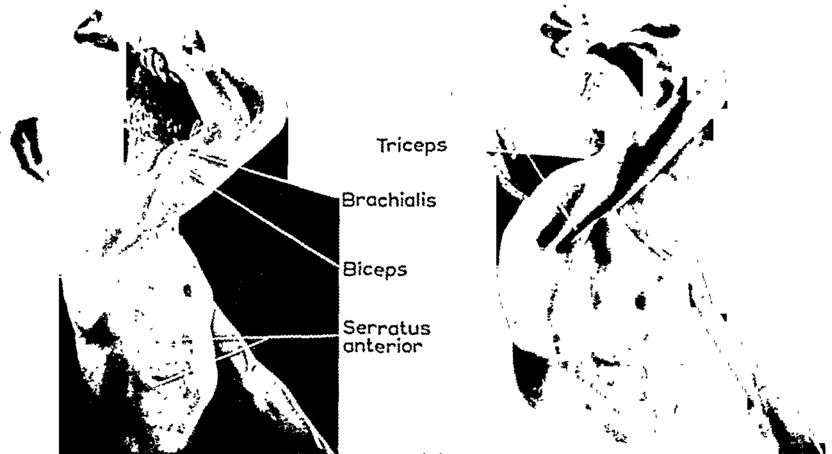


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ground, leg muscles pulling from the foot increase tension upon the fascial stocking ensheathing the leg muscles.

In walking, the initial forward inclination of the trunk is made by relaxing the tension of the hamstring and calf muscles in both limbs. As the right foot leaves the ground, the trunk is balanced by a tilt to the left. The right limb swings forward flexing at knee and hip and extending as the heel touches the ground. The impetus in the push-off in walking, running, and jumping is given by the propelling limb extending at the knee and hip and plantar flexing at the ankle. The left limb now propels, the left heel is raised by the calf muscles and the weight, at first carried by the whole foot, is shifted forward. The final take-off is from the metatarsal pad region, especially that of the great toe. The final impetus is derived from the flexors of the toes. Alternately during walking, at each push-off, the extending limb pushes downward and backward and the body is propelled upward and forward to be caught as its trajectory begins to fall by the opposite limb swinging forward in front of the body.

E. Muscle Power, Range, and Speed of Movement

The muscle with the greater number of fibers is the more powerful, and the longer the fibers in a muscle the greater its range of movement. The long narrow strap-like sartorius with its parallel fibers effects a wide movement, but it does not have the power of a short multipennate type like the deltoid. As the strength of a muscle depends upon the sum of the areas of cross section of the separate fibers, weight for weight the muscle with the greater number of fibers is the stronger.

Speed is an intrinsic quality varying with individual muscles, inherent in the biochemical and physicochemical makeup of the muscle and not dependent alone upon the nerve supply. The smaller the structure to be moved, such as the eyeball, the more rapid its muscles, compared with the heavy gluteus maximus muscle moving the trunk and lower limb. According to Sotavalta, (1947) the wing muscles of a gnat beat at the rate of 1/1000 of a second while the sea anemone takes 4 minutes to contract (Pantin, 1956).

Muscles are accurately designed for their usual work. A compromise exists between power, speed, and range, according to the differences in number of fibers, their length, intrinsic speed, the position in relation to joints, and the weights to be moved.

The finest athletic achievements depend upon a remarkable effi-

ciency in timing the various associated movements to a nicety. A. V. Hill (1951) cites the record throw of a cricket ball which leaves the fingers at a rate of about 82 miles per hour, a result achieved by the summation of the velocity of each successive part of the body's movement added to that of the previous parts—a principle probably more readily appreciated in the parallel of the cracking whip where the ultimate velocity of the tip breaks through the sound barrier.

F. Isometric and Isotonic Contraction

When a muscle fails to lift a weight beyond its strength, although its tension is maximal, its length is unaltered and its condition is described by the somewhat contradictory terms isometric contraction.

In isotonic contraction, the length varies but the tone remains the same.

G. Voluntary Movement

Skeletal or striated muscle is frequently termed voluntary muscle but this is a figure of speech because only the movement is voluntary. We have no freedom in selecting the muscle which carries out the movement. Indeed, there are usually several muscles involved in a definite pattern of activity and timing and we are incapable of altering the pattern or increasing or decreasing the numbers involved by even one member. In the simple action of jutting out the thumb laterally, the muscles on the front and back of the ulnar side of the forearm are seen and felt contracting to prevent the whole hand moving in the same direction. Even if the thumb muscles are paralyzed, the ulnar muscles contract the moment the movement is attempted.

According to the parts they play—principal or supporting in a movement—muscles are described as prime movers, antagonists, fixation muscles, and synergists.

H. Prime Movers and Antagonists (Figs. 3—10)

The prime movers are the principal actors. They actively bring about the desired movement. The antagonists must relax to allow the prime movers to operate. Despite its name, the antagonist assists the prime mover. It is as important in a good system of training to secure full relaxation as well as full contraction of a muscle. Indeed, in some people, the pectoral muscles are so tight that the arms cannot be raised erect, and calf muscles in most people prevent the full amount

ground, leg muscles pulling from the foot increase tension upon the fascial stocking ensheathing the leg muscles.

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of dorsiflexion at the ankle. When the prime movers are contracting, the antagonists, although not stimulated, are immediately ready to steady the part as a guy rope in securing precision of movement. As an act of volition, of course, we can set in firm tension both prime movers and antagonists at the same moment just as a professional strong man accentuates his muscles in a pose for exhibition.

I. Fixation and Articular Muscles

Fixation and articular muscles fix the base upon which the movement carried out by the prime movers is made. During movements of the upper limb, the scapula is steadied by fixation muscles such as serratus (Figs. 3 and 4), and the head of the humerus is kept in position on the scapula by small articular muscles which prevent the head of the humerus from sliding when the arm is raised—much in the way that a man places his foot upon one end of a long ladder while his companion raises the other end. Anyone who has had the exasperating experience of trying to raise a ladder too long for him single handed will have a keener appreciation of the value of a fixation muscle. The rectus abdominis muscles become painful in repeating the exercise of raising both legs with the body supine, not because they act directly on the limbs but because they must fix the pelvis in counteracting the heavy leverage of the limbs.

J. Synergists

Special instances of fixation muscles, controlling movement at proximal joints and thereby enabling prime movers to act upon a distal joint, illustrate muscular synergy as explained in the legend to Figs. 7 and 8. However, both fixation muscles and synergists prevent loss of power and there is no rigorous distinction between them.

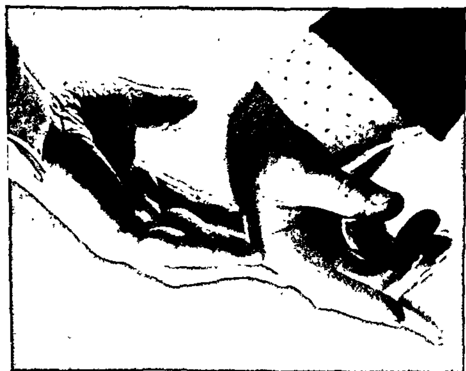
K. The Group Action of Muscles

Prime movers, antagonists, fixation, and synergist muscles perform

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Figs. 7 and 8 Muscular synergy or combined action. The firmly clenched fist is always bent backwards (extended) at the wrist, but the fingers lose their grip and open when the wrist is forcibly bent forwards (flexed), a method of compelling an assailant to drop his weapon. The extensor muscles cannot stretch far enough to allow the flexors full action both at wrist and fingers. Accordingly these two rival muscle groups co-ordinate in a precise synergic or combined action whereby the extensors control, or fix, the proximal wrist joint while the flexors clench the fingers at the distal joints. Reproduced from "Living Anatomy" by R. D. Lockhart (1959) (Courtesy of Faber & Faber, London; Oxford University Press, New York).





shortened with the result that full dorsiflexion of the foot can no longer be obtained. In marked cases, the person may experience painful discomfort in walking with heelless shoes. This is one of the causes of flat foot and is easily remedied by resuming the customary heel. In the above examples the muscles are passively insufficient, a condition already discussed in Section II, B.

N. Associated or Cooperative Action

The terms associated or cooperative action may be quite properly applied to the group action of prime movers, antagonists, fixation muscles, and synergists, but usually it refers to more remote muscles which act in unison. For example, the head and often the trunk turn in the same direction as the eyes. In fact, it requires a little premeditation if not practice to move the eyes without automatically turning the head in unison.

If the pads of the thumb and forefinger of the right hand are pressed very lightly together the other three fingers can be easily moved to and fro by the left hand, but if the thumb and forefinger are tightly compressed the other fingers become immobile. Again, tight closure of the eyes by the orbicularis oculi muscles is accompanied by a faint drumming in the ear as the stapedius muscles contract in unison (Gowers, 1896).

O. Muscles Never Act in Diametrically Opposed Movements

The fact that a muscle happens to be favorably placed for the execution of a movement and even the fact that it does so under electrical stimulation provide no guarantee whatever that, in the normal state, the muscle does carry out this movement. It has been stated that the sternomastoid, when the head is bent backwards, is able to induce further extension. But Beevor demonstrated a case of paralysis of the extensor muscles of the back of the neck in which, with the patient supine, and the head well back in good position to be further extended, the patient actually relaxed the sternomastoid when asked to extend the head.

The pectoralis major, a most interesting muscle, has two parts, a clavicular and a sternocostal (Fig. 9). Both act together in adduction of the arms. But in pulling down the arm against resistance, only the sternocostal part is active; the clavicular part is inactive although well placed to depress the erect arm (Fig. 9). In raising the arm, however,

together in most movements. Their cooperation is described as group action. But the term is also applied to a group of prime movers or antagonists, where each group comprises a rival team. If the surgeon transplants the tendon of an antagonist to take the place of a paralyzed prime mover, then the transplanted muscle will continue to act with its old team in the accustomed pattern of reciprocal activity until a cooperative patient re-educates the muscle (Dunn, 1920).

L. The Dual Action of Muscles

Dual action of muscles is exemplified by a muscle taking part in two distinct, but not opposite, movements. The biceps is both a flexor of the elbow and a supinator of the forearm, and, as the result of cortical injury, the power of one movement may be lost and the other movement retained. In conjugate movements of the eyeballs the medial rectus muscle of each eye may act with the lateral rectus of the opposite eye but fail to act in convergence of the eyes with the opposite medial rectus (Beevor). Apart from voluntary movements, as in the above examples, muscles may also be engaged in bilateral involuntary movements. Latissimus dorsi in its unilateral voluntary movement adducts the arm against resistance, but both latissimus dorsi muscles have also an involuntary bilateral action in coughing or sneezing, an expiratory function easily felt by placing the hands against the lower ribs and coughing. If the clinician finds the bilateral action retained and the unilateral lost, then he has valuable evidence that the lesion is cerebral and not spinal or peripheral (Beevor).

M. The Ligamentous Action of Muscles

The ligamentous action of muscles is well seen in the inability of the hamstring muscles to relax sufficiently to allow full flexion at the hip joint when the knee is extended and accounts for inability to touch the toes and to maintain or even attain the high kick position. Pectoral muscles may be unable to stretch enough to allow the arms to be raised erect. To get the arms erect, the person bends the vertebral column backwards. Again, the calf muscles, always much heavier than the anterior leg muscles, a feature characteristic of man, tend to draw up the heel producing plantar flexion of the foot when the body is recumbent. The wearing of heels, even in men's shoes, has the same effect in ultimately allowing the calf muscles to become permanently

two minutes when the circulation is prevented, and within another minute impossible (Merton, 1956).

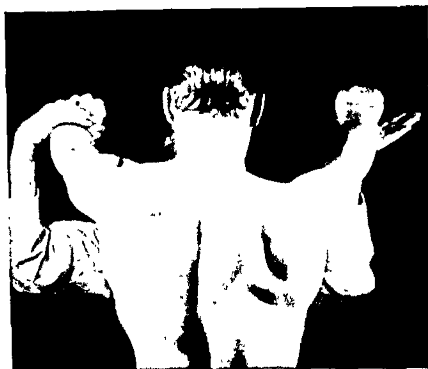


Fig. 10. [illegible] resistance, while on the left the trapezius is firmly contracted in raising the left arm against resistance.

At the beginning of this chapter the importance of determining the action of muscles in the living body was stressed. In conclusion it must again be reiterated that despite the scalpel, electrical stimulation, and electromyography, the method of reinforcing the action of muscles by resisting the patient's movements, so admirably demonstrated by Charles Beevor, is simple, immediate, and provides both student and experienced physician with literally first hand information.

References

Beevor, C. E. (1904). "The Croonian Lectures on Muscular Movements and their Representation in the Central Nervous System." Adlard, London; (1951). Edited and Reprinted for publishers of "Brain" Macmillan London.

Brash, J. C. (1955). "Neurovascular Hila of Limb Muscles." Livingstone, Edinburgh.

Duchenne, G. B. A. (1867). "Physiologie des Mouvements démontrée à l'aide de

the clavicular part is active and the sternocostal relaxed (Fig. 9). This is an instance where a knowledge of the two attachments, origin and insertion, of a muscle is no guide to the action.



Fig. 9. Actions of pectoralis major muscle. The right arm is raised against resistance and the left arm lowered against resistance. The clavicular part of pectoralis major is active in the right arm and inactive in the left arm. The sternocostal part is inactive in the right arm, so that the pencil may be depressed into the muscle, and active in the left arm so that the pencil cannot be impressed into the muscle. The upper fibers of trapezius, the anterior fibers of deltoid, and the biceps are seen active on the subject's right side and flaccid on his left.
Reproduced from "Living Anatomy" by R. D. Lockhart (1959) (Courtesy of Faber & Faber, London; Oxford University Press, New York).

P. Muscular Fatigue

The fact that an air cuff when applied to stop the circulation in a limb exhausted by heavy exercise prevents the recovery of strength until the circulation recommences, seems to indicate that the muscle and not the nervous system is the site of the fatigue. The act of writing, an intricate and skilled but light exercise, becomes difficult within

CHAPTER II

The Microanatomy of Muscle

E. W. Walls

I. VOLUNTARY, SOMATIC, OR SKELETAL MUSCLE

A. General Architecture

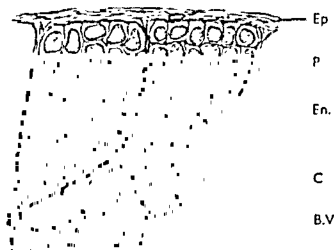
In considering the detailed structure of voluntary muscle, it is helpful to begin by examining a section through a whole muscle, such as the superior rectus muscle of the eyeball (Text Fig. 1, and Plate I, Fig. 1). Surrounding the muscle there is a connective tissue sheath called the epimysium, from the deep surface of which septa pass into the muscle at irregular intervals. These septa, which constitute the perimysium, invest bundles (fasciculi) of muscle fibers, the bundles being of somewhat angular outline in section and of different sizes. Subdivision of the larger bundles into two or three successively smaller bundles is frequently observed. The name perimysium is retained for the connective tissue surrounding the lesser bundles. Finally, from the perimysium very delicate extensions of fine connective tissue called the endomysium pass in to surround each muscle fiber.

The size of the fasciculi composing a muscle determines its texture.

l'Experimentation electrique et de l'Observation Clinique." J.–B. Baillière et [illegible] Translated and edited by E. B. Kaplan.

D[illegible]
Floyd, W. F., and Silver, P. H. S. (1955). *J. Physiol.* **129**, 184.
Gowers, Sir W. (1896). *Lancet* **ii**, 1357.
Haines, R. W. (1934). *J. Anat.* **69**, 20.
Hellebrandt, F. A. (1938). *Am. J. Physiol.* **121**, 471.
Hill, A. V. (1951). *Lancet* **ii**, 261, 947.
Joseph, J., and Nightingale, A. (1952). *J. Physiol.* **117**, 484.
Joseph, J., and Nightingale, A. (1954). *J. Physiol.* **126**, 81.
Joseph, J., and Nightingale, A. (1956). *J. Physiol.* **132**, 81.
Joseph, J., Nightingale, A., and Williams, P. L. (1955). *J. Physiol.* **127**, 617.
Lockhart, R. D. (1959). "Living Anatomy, A Photographic Atlas of Muscles in Action and Surface Contours." 5th ed. Faber and Faber, London.
Lockhart, R. D., and Brandt, W. (1938). *J. Anat.* **72**, 470.
Merton, P. A. (1956). *Brit. Med. Bull.* **12**, 219.
Pantin, C. F. A. (1956). *Brit. Med. Bull.* **12**, 199.
Sotavalta, O. (1947). *Acta entomol. Fennica* **4**, 177.
Walshe, F. M. R. (1951). In Foreword to reprint of Croonian Lectures by Beevor, C. E.
Winslow, J. B. (1732). "Exposition Anatomique de la Structure du Corps Humain," Desprez, G., Paris; (1734) Translated by G. Douglas, Betterworth and Hitch, London; (1743) G. Douglas, R. Ware (and others) London.

Thus in muscles capable of very finely judged movements, for example the ocular muscles, the texture is fine, whereas in muscles which perform movements demanding less precision but greater power (e.g., those of the buttock) the fasciculi are much larger and the texture is correspondingly coarse. It is of interest to note that this distinction



Text Fig. 1. Diagram of the connective tissue framework of a voluntary muscle based on part of Plate I, Fig. 1. From the epimysium (Ep), which surrounds the whole muscle, septa pass inwards to surround variously sized bundles of fibers. The connective tissue sheaths of the bundles constitute the perimysium (P) and from it *delicate strands continue between the individual muscle fibers as the endomysium* (En.). Larger blood vessels (B.V) are present within the perimysium, and capillaries (C) lie between the individual muscle fibers. Compare with Plate I, Figs. 3 and 7.

Fig. 1. Cross section of superior rectus muscle of human adult aged 65 years, to show the general structure of a voluntary muscle. Compare with Text Fig. 1. Hematoxylin and eosin. Magnification: × 16.

Fig. 2. Two fibers from a thigh muscle of a rat cut in longitudinal section and stained with Heidenham's iron hematoxylin. The fibers appear to be at different stages of contraction. In the upper one the Z bands may be distinguished and in the lower the H bands. Magnification: × 1050.

Fig. 3. Tongue of cat in which voluntary muscle fibers can be seen cut in cross section, longitudinally, and obliquely. There are numerous capillary blood vessels present between the muscle fibers. Ehrlich's hematoxylin and Van Gieson. Magnification: × 240.

Fig. 5. Cross section of superior rectus muscle of human adult aged 65 years. For comparison with Fig 6. Hematoxylin and eosin. Magnification: × 230.

Fig. 6. Cross section of omohyoid muscle from same subject as Fig. 5. Hematoxylin and eosin. Magnification: × 230.

Fig. 7. Reticular fibers in endomysium of human voluntary muscle. Silver impregnation. Magnification: × 1050.

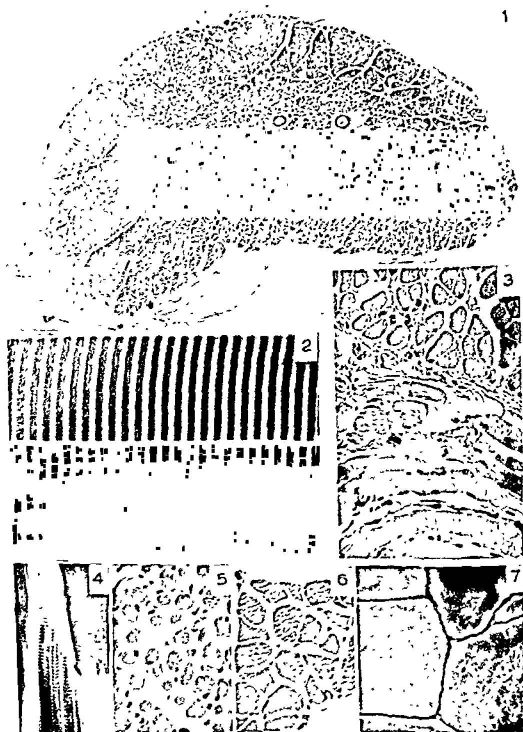


PLATE 1

of the muscle will be considered below in relation to the muscle-tendon junction. First, however, the mechanical role which connective tissue plays within the substance of the muscle must be discussed. To some degree the sheaths which it forms both for the individual muscle fibers and for the muscle fasciculi (interconnected as they are with one another) must regulate and control the extent of contraction. It is of interest that the proportion of connective tissue present is greater in muscles which are capable of finely graded movements (Fernand, 1949). Further, as Banus and Zetlin (1938) have shown, the initial tension which develops in a relaxed muscle when it is extended is due solely to its connective tissue content.

In a later chapter, the sequence of events in regeneration of voluntary muscle will be described, but it is appropriate to mention here that the endomysial sheaths of the individual fibers, in other words their investing connective tissue tubes, serve as guides for the new fibers which sprout out from the stumps of the old.

C. The Muscle Fibers of Voluntary Muscle

1. *Fiber shape*

Within its connective tissue framework, the tissue of voluntary muscle is formed by independent, cylindrical fibers which are in fact elongated, multinucleated cells. This last character may allow each fiber to be regarded as a syncytium but actually each is a structural unit with no other evidence of a composite cellular origin—if indeed it does arise by the fusion of many separate cells.

In mammals it is rare to find the fibers anastomosing with their neighbors but in invertebrates this is not uncommon. Generally speaking, too, voluntary muscle fibers do not branch, but in the tongue of the frog the muscle fibers divide into numerous branches as they approach their insertion to the mucous membrane. This has also been observed in other animals, particularly those with freely mobile tongues, and to a lesser extent in man. In mammals, those fibers of the facial musculature which are attached to the skin show this same feature.

The shape of the fibers of striated muscle has been studied by many workers, who by dissecting individual fibers established that they were elongated elements, more or less tubular, but of varying appearance. Bardeen (1903), investigated in this way the external oblique muscle of the abdomen in the dog, rabbit, and man, and classified the fibers

between muscles of fine and coarse texture is further instanced by differences in the sizes of their motor units.

In muscles which lie immediately under the deep fascia, the relation which the epimysium bears to the tissue varies in different parts of the body. Thus where the deep fascia is a well-developed layer, as in the thigh, loose areolar tissue is present between the fascia and the epimysium of the subjacent muscles; in regions where the deep fascia is more delicate in texture, however, it is not clearly to be distinguished from the epimysium.

In these sites where muscles take origin from the overlying deep fascia, it is not possible to demonstrate epimysium. Here the process of cleaning a muscle, which elsewhere simply means the removal of epimysium whereby the run of the muscle fibers may be revealed, is tedious and at best unsatisfactory involving as it does some cutting across of the muscle fibers.

B. The Connective Tissue Component of Voluntary Muscle

The amount of connective tissue relative to muscle fibers is much greater in some muscles than in others, a fact which largely explains why some cuts of meat are tougher than others. The lower the collagen content the more tender is the cut, and vice versa. In lean meat the proportion of the total protein formed by collagenous tissues ranges from 3 to 30%.

The elements composing the connective tissue of muscle are collagen fibers, reticular fibers, elastic fibers, and several varieties of cells such as fibroblasts, histiocytes (macrophages—also called clasmatocytes or resting wandering cells), and fat cells. The amount of elastic tissue present varies with the type of action of the muscle, being greatest in those muscles which are attached to soft parts, e.g., the muscles of the tongue and of the face. It has, moreover, been shown by Bucciante and Luria (1934) that there is an increase in the elastic tissue component of the superior rectus muscle with aging. The connective tissue between the individual muscle fibers consists of reticular fibers and of fine collagen fibers; often very scanty in amount, it conveys the blood capillaries and the smallest branches of the nerves. The larger blood vessels and nerves lie within the perimysium between adjacent fasciculi, a situation in which muscle spindles are also commonly found. The manner of attachment of the connective tissue component to the tissue which serves as the origin or insertion

1932), and rabbit (Graf, 1917). Third, all observers are agreed that in any cross section of a muscle the range of fiber diameter is very wide. It could be argued that the thin fibers seen are in fact the tapering ends of thick fibers but Schiefferdecker (1909) thought this played only a small and unimportant role when comparison is made between the caliber of the fibers of two different muscles. He therefore concluded that muscle fibers of different cross section were present in the same muscle, and this is now the majority view. Huber (1916) considered dissection of separate fibers the method most likely to solve the problem, and Lindhard (1926) did this in teased preparations of frog muscle and showed thick and thin fibers lying side by side.

The spatial distribution of fibers of different diameter seen in cross sections of muscle has received little attention. Garven (1925) noted that the smaller calibered fibers were situated at the periphery and Herrick (1902) made a similar observation in the extrinsic eye muscles of fishes. From a study of the many photographs and tracings of muscle cross sections in the literature, Fernand (1919) states that thick and thin fibers appear side by side in all areas of the muscle cross section, but that generally the largest fibers are situated in the depth of the muscle. In his own work on the rabbit Fernand found three groups of muscles: (1) those with large fibers at the periphery of their cross section, (2) those with large fibers in the depth of the cross section, and (3) those with no characteristic spatial distribution.

Many attempts have been made to establish the factors which may be correlated with the varying diameters of muscle fibers. Thus there is some support for the idea that longer fibers have a larger diameter (Tsuruyama, 1937), but there is no direct correlation. A quotation from Fernand (1949) puts the matter well: "Thus in the normally developed muscles of the rabbit, there appears to be no correlation between the length of the fibres in the different muscles and the diameter of the fibres. However, the observation that the fibres of the largest calibre are found in the muscles with the longest fibres, as for instance the biceps femoris and semimembranosus suggests that longer fibres have the potentiality for developing a greater girth than shorter fibres." In man Kohashi (1937) found that the most powerful muscles have the thickest fibers, and Oshima (1938) reached the same conclusion for the cat. Fernand agrees that there is a correlation but points out that other factors such as the number of muscle fibers

into three categories: (a) fibers of nearly cylindrical shape, with conical ends, running from origin to insertion; (b) fibers of fusiform shape, with fine tapering extremities ending within the belly of the muscle; and (c) fibers of conical shape, the broad base being attached to a tendon and the other end terminating in a fine attenuated extremity. Bardeen's findings confirmed those of several earlier workers and later were to be confirmed in turn by Huber (1916) for the rabbit and Lindhard (1926) for the frog.

When muscle fibers are sectioned transversely the shape of their cross sections is oval or spherical if cut when fresh, but irregularly polyhedral if cut after fixation; the difference is due to shrinkage both of their surrounding connective tissue and of the muscle fibers themselves during fixation. It is of interest that Watzka (1939) found less shrinkage in the fibers of the wild rabbit than in those of the tame one, and less in the "red" fibers of the domestic animal than in the "white" fibers of the same animal; and further, that there was more connective tissue in the muscles of the wild variety than in the tame variety, and more in the red muscles than in the white ones of the latter. In brief, the fibers of muscles with more connective tissue shrank less. The diameters of isolated, freely suspended muscle fibers from the semitendinosus muscle of the frog were measured by Buchthal and Knappeis (1946) in two planes at right angles to each other. Even under these conditions when external pressure plays no part it was found that the cross section of the fiber is usually somewhat oval.

2. *The Diameter of Voluntary Muscle Fibers*

There is a considerable range in the diameter of different voluntary muscle fibers, 10–100 μ commonly being accepted. First, it may be said that their size differs in the major animal classes, as shown by Bowman (1840), Mayeda (1890), and Schwalbe and Mayeda (1891). Using a standard method of fixation Mayeda dissected the fibers of a large variety of muscles, especially those which he considered to be homologous, and obtained measurements which showed that the fiber diameter descends in this order: fish, toads, reptiles, mammals, and birds. Second, the fibers in one muscle may be generally larger than those of another muscle in the same animal. Thus the difference in the mean fiber diameter of muscle samples from different parts of the body, or from different muscles of the same limb, have been demonstrated in man (Halban, 1893), sheep (Hammond and Appleton,

female rats kept under identical conditions. It may be that this matter should be further investigated.

3. *The Length of Voluntary Muscle Fibers*

It is generally agreed that in short muscles the fibers may run from the origin of the muscle to its insertion—that is to say the fibers and muscle belly may be coextensive. For example, in a muscle such as the tensor tympani the fibers are only a few millimeters long. With regard to the length of the fibers in longer muscles, the consensus of opinion seems to be that the fibers do not run from end to end of the muscle. Nevertheless, there is evidence that in long muscles with parallel-running fibers such as the sartorius, this may be the case—at any rate in man. The classification of fibers by shape, drawn up by Bardeen, to which reference has been made, shows that in the external oblique muscle some fibers do run from end to end while others have one or both extremities terminating within the muscle. The findings made in the human sartorius will now be considered. In an adult human sartorius muscle 52 cm. long, Lockhart and Brandt (1938) were able to isolate fibers 34 cm. in length, and even at that the ends were broken across. These authors also state that in a fetus with a sartorius 5 cm. in length the fibers when isolated by teasing could be seen to run the complete length of the muscle; and that this was also the case with the semitendinosus. They also found that in adult eye muscles the fibers run all the way. Of several earlier workers who dissected the human sartorius, Felix (1887) obtained the longest fibers, but his figure of 12.3 cm. falls far short of that given by Lockhart and Brandt. The conflict in findings must be related to the technical difficulties involved in this type of investigation.

In a rabbit's thigh muscle Huber (1916) teased out a fasciculus 3.4 cm. long, comprising 26 muscle fibers, and found only 1 fiber passing from end to end; of the remainder, 10 fibers reached one tendon end, 12 reached the other, and 3 were spindle-shaped reaching neither end. He also observed that the disposition of the fibers was such that their fine filamentous intra-fascicular endings were usually applied to the thicker parts of neighboring fibers. Van Harreveld (1947) states that in the rabbit sartorius only about two-thirds of the total number of muscle fibers present in the muscle appear in any single cross section, and that in the length of the muscle there are 3 separate fibers arranged in series. It is quite clear that the longer muscle fasciculi tend to have

contained in the muscle and the functional axis of the muscle also contribute to the final determination of fiber caliber.

Concerning the part which muscle size may play, the evidence is conflicting. In the sheep, Hammond and Appleton (1932) found that the actual size (weight) of the muscle has "little or no effect on the size of the fibre," but in the muscles of man Halban's work does suggest a link, although not a constant one, between muscle and fiber size. His illustration contrasting fiber size in the superior rectus and gluteus maximus muscles is reproduced in Text Fig. 2. Body size in farm animals cannot be correlated with fiber diameter, but nutritional



TEXT FIG. 2. Diagram of the difference in fiber size which may be met with in two muscles of the same subject; (a) ocular muscle, (b) gluteus maximus. Magnification: × 340. (after Halban). Compare with Plate I, Figs. 5 and 6. In one fiber of group (b) Cohnheim's areas have been indicated.

state is apparently of importance; not only does inanition lead to a decrease in fiber caliber, but full fed steers have larger fibers than those rough fed (Robertson and Baker, 1933), and pigs which enjoy better feeding in their first 16 weeks achieve a bigger fiber diameter when adult than control animals (McMeekan 1941). The facts that muscle fibers increase in diameter from birth to maturity and also in response to exercise are well known, but the influence of sex on fiber size is less certain. It is generally accepted that the fibers of the same muscles are larger in human males than females (Bowman, 1840; Schwalbe and Mayeda, 1891), and in the sheep Hammond and Appleton (1932) found the ram to have larger fibers than the ewe. However, Elliot *et al.* (1943) could not detect any difference in fiber size between male and

fact to the endomysium. Wang (1956) suggests that in describing the sarcolemma, which he regards as a composite structure, clarity would be ensured by referring to the sarcolemma proper or true sarcolemma and to the sarcolemmous envelope. The latter he regards as a fibrous envelope having a greater structural affinity with the sarcolemma than with the endomysium. With regard to its physical properties which will also be considered in a later chapter, it can be said here, first, that it is possessed of remarkable electrical properties and, second, that it appears to be elastic. Concerning its mechanical properties, Ramsay and Street (1940) found that following the production of retraction clots in single muscle fibers the empty sarcolemma reached to stretch in the same way as the intact fiber; that is to say, the same increase in load produced the same percentage elongation, a result which suggests that the resting tension of the muscle fiber is governed by the sarcolemma alone. This finding of Ramsay and Street brings to mind the observation made by Banus and Zetlin (1938) that the connective tissue sheath of a whole muscle, when dissected free, gave the same tension-length curve as the whole muscle. However, Sichel (1941) obtained a quite different result finding that the elongation of empty lengths of sarcolemma averaged more than twice that of intact regions. If correct, this would mean that the intact muscle fiber would resist extension by a tension fully twice that of the sarcolemma alone.

According to Buchthal (1942), it is not possible to measure the true resting tension of the sarcolemma in the region of a retraction clot since it is already elongated. Barer (1948) would allow the force of this argument when the clot is large and hard, but points out that it is possible to produce empty lengths of sarcolemma without such clots and without any apparent lengthening.

How the sarcolemma serves to transmit the contractile force of the muscle fibers to the connective tissue and muscle tendon will be considered later; that its elasticity may account for the appearance of "active" relaxation in single muscle fibers is discussed by Fenn (1945).

5. *The Nuclei of Striated Muscle Fibers*

Each striated muscle fiber possesses many nuclei and in a fiber some centimeters long there may be several hundred. In the great majority of fibers of human and mammalian muscle, the nuclei lie at the surface of the fiber immediately under the sarcolemma, surrounded by a

longer fibers, even though the proportion of these which end intrafascicularly is not yet agreed. The great length of the fibers in a muscle like the sartorius and their arrangement in longitudinally disposed fasciculi is typical of muscles which act over freely movable joints, allowing as it does the maximum displacement of the parts to which they are attached. The amount of this displacement corresponds of course to the degree of shortening which it is possible for a muscle fiber to undergo during contraction; for human muscle Haines (1934) states that shortening to 57% of its length when fully stretched can occur.

4. *The Sarcolemma*

Covering the surface of each muscle fiber and closely and completely investing it is a thin membrane called the sarcolemma. Only 0.1 μ in thickness, it forms a resistant sheath enclosing the soft protoplasmic contents of the fiber. During contraction, the sarcolemma follows the changing shape of the protoplasmic contents. Forming as it does "a tubular membranaceous sheath of the most exquisite delicacy" (Bowman, 1840), it is too thin to be studied in ordinary fixed and stained preparations. It is especially well seen in fish and amphibia, in which it is thicker and stronger than in the mammals (Schafer, 1912). Electron microscopy apart, the best method of demonstrating the sarcolemma is to examine a few roughly teased fresh striated muscle fibers in Ringer's fluid (Barer, 1948). Where the fibers have been damaged, retraction clots form (for details of their formation see Speidel, 1939), and at the side of each an empty space is left within the intact sarcolemma which can then be studied free of muscle substance Several other ways of achieving the same object are also mentioned by Barer. Thus anything which causes a violent contraction of the muscle fiber—e.g., treatment with caffeine, nicotine, or saponin, may cause the sarcolemma to bulge away from the contracted muscle substance; colloidal swelling agents such as dilute acids or alkalies may produce swelling of the muscle substance with subsequent rupture of the sarcolemma, while treatment with trypsin digests the muscle substance leaving the sarcolemma seemingly intact.

Thus revealed, the sarcolemma appears as a homogeneous, apparently structureless membrane It might be proper to remark at this point that in the past confusion has arisen from the term sarcolemma having been applied to connective tissue elements which belong in

in sarcoplasm. This point will be returned to when red and white muscle fibers are considered.

The advent of the electron microscope has resulted in a much more detailed understanding of sarcoplasmic organization [see Bennett (1955, 1956) and Bennett and Porter (1953)]—but it tends to be forgotten, and Bennett has done well to remind us, that Retzius and Cajal, among others, had observed with the light microscope extremely delicate longitudinal and transverse filaments within the interstitial substance between the myofibrils. This sarcoplasmic reticulum was also described and indeed figured by Heidenhain. Besides confirming many of the findings of these old masters, Bennett and Porter have added many more. Quite briefly, it may be said here that the sarcoplasmic reticulum is entirely a sarcoplasmic component not being seen within the myofibrils. It has connections with the Z bands, less regularly with the M bands, and is also found in close association with the mitochondria. It has been suggested that the sarcoplasmic reticulum functions as a means of exchange of substances between the myofibrils and the sarcoplasm during contraction and relaxation. Equally attractive is the postulate that it serves as a system for the rapid conduction of the excitatory impulse to contraction both transversely across the fiber and longitudinally.

7. *The Myofibrils*

The name "striated" as applied to muscle refers to the cross banding which is such a striking feature of voluntary (and see later cardiac) muscle when its fibers are examined microscopically. However, another type of striation is also noticeable, viz., longitudinal, which is due to the longitudinal disposition within the sarcoplasm of the fiber of its constituent myofibrils, also called sacrostyles. In a cross section of a muscle fiber, these elements can be seen as fine dots which may be uniformly distributed in the sarcoplasm or gathered into groups, the polygonal areas or fields of Cohnheim (1865), separated by greater amounts of clear sarcoplasm. Teasing of a muscle fiber after treatment with, say, chromic acid, will split it up into bundles of myofibrils and in favorable cases individual fibrils can be separated off In Plate I, Fig. 4, the end of a muscle fiber cut in longitudinal section which has frayed during cutting is seen, the result being that some of its constituent myofibrils appear clearly. That myofibrils exist in living muscle and are not caused to form by subsequent

zone of protoplasm. It follows that opposite the nucleus the fibrils (shortly to be described) do not quite reach to the inner surface of the sarcolemma. The nuclei are of an ovoid form, elongated in the long axis of the fiber, and average 8–10 μ in length although extremes of 5–17 μ are met with (Sobotta, 1930). In the fresh state the nuclei are difficult to see but in fixed and stained preparations they are readily demonstrated. Along the fiber their distribution is fairly regular, but toward the tendinous attachment they become more numerous and are more irregularly distributed. The position of the nuclei beneath the sarcolemma, i.e., hypolemmal, which is usual in adult human muscle, differs from that found in the embryo in which the nuclei occupy a position in the middle of the fiber. In many lower vertebrates (fishes, amphibia, and reptiles), the nuclei lie within the fiber, either centrally or eccentric, but seldom just under the sarcolemma. This is commonly said also to be the case for the so called red muscles of mammals, but this point will be discussed later.

It should be emphasized that the usual position of the nuclei on the periphery of the fiber is best appreciated when transverse sections through muscle fibers are examined. In longitudinal section, many nuclei do appear in this position, but clearly those nuclei which lie on the upper or lower surface of longitudinally disposed fibers will appear as if they were in the middle of the fibers.

6. *The Sarcoplasm*

It is a convenient simplification to regard striated muscle fibers as being composed of four main constituents, viz. sarcolemma, nuclei, sarcoplasm, and myofibrils. Of these, the last two remain to be discussed. Sarcoplasm is commonly regarded as undifferentiated protoplasm of a semi-fluid consistency, in which the myofibrils are embedded. The observations of Speidel (1939) on living muscle suggest that in normal fibers the sarcoplasm and the myofibrils are in the gel state and that following injury this may change to that of a sol. In an ordinary histological preparation showing the muscle fiber cut in transverse section, it can be seen that within the fiber the space bounded by the sarcolemma is not completely occupied by the myofibrils; the residual space is presumably filled by sarcoplasm. Not all fibers show the same relative amount of sarcoplasm to myofibrils and Knoll (1891) who investigated the matter very thoroughly divided fibers into those which were rich and those which were poor

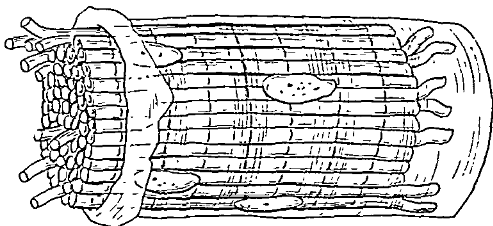
in sarcoplasm. This point will be returned to when red and white muscle fibers are considered.

The advent of the electron microscope has resulted in a much more detailed understanding of sarcoplasmic organization [see Bennett (1955, 1956) and Bennett and Porter (1953)]—but it tends to be forgotten, and Bennett has done well to remind us, that Retzius and Cajal, among others, had observed with the light microscope extremely delicate longitudinal and transverse filaments within the interstitial substance between the myofibrils. This sarcoplasmic reticulum was also described and indeed figured by Heidenhain. Besides confirming many of the findings of these old masters, Bennett and Porter have added many more. Quite briefly, it may be said here that the sarcoplasmic reticulum is entirely a sarcoplasmic component not being seen within the myofibrils. It has connections with the Z bands, less regularly with the M bands, and is also found in close association with the mitochondria. It has been suggested that the sarcoplasmic reticulum functions as a means of exchange of substances between the myofibrils and the sarcoplasm during contraction and relaxation. Equally attractive is the postulate that it serves as a system for the rapid conduction of the excitatory impulse to contraction both transversely across the fiber and longitudinally.

7. *The Myofibrils*

The name "striated" as applied to muscle refers to the cross banding which is such a striking feature of voluntary (and see later cardiac) muscle when its fibers are examined microscopically. However, another type of striation is also noticeable, viz., longitudinal, which is due to the longitudinal disposition within the sarcoplasm of the fiber of its constituent myofibrils, also called sacrostyles. In a cross section of a muscle fiber, these elements can be seen as fine dots which may be uniformly distributed in the sarcoplasm or gathered into groups, the polygonal areas or fields of Cohnheim (1865), separated by greater amounts of clear sarcoplasm. Teasing of a muscle fiber after treatment with, say, chromic acid, will split it up into bundles of myofibrils and in favorable cases individual fibrils can be separated off. In Plate I, Fig. 4, the end of a muscle fiber cut in longitudinal section which has frayed during cutting is seen, the result being that some of its constituent myofibrils appear clearly. That myofibrils exist in living muscle and are not caused to form by subsequent

treatment with chemicals seems no longer in doubt (Jordan, 1933; Speidel, 1939). Thus, in the tails of young frogs and salamander tadpoles, Jordan studied striated muscle under high magnification and was fully satisfied that myofibrils and cross striations exist in living muscle fibers. Tissue culture studies have given mixed results, but Hogue (1937) demonstrated cross striated myofibrillae in living cultures of cardiac muscle cells. Microdissection of fresh muscle fibers as a means of demonstrating myofibrils is exceedingly difficult (Barer, 1947) the trouble being that in the living fiber the stickiness of the sarcoplasm tends to cause the myofibrils to join together in irregular



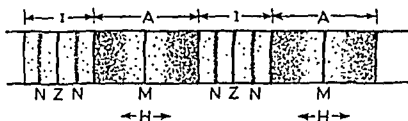
TEXT FIG. 3. Diagrammatic representation of the structure of one voluntary muscle fiber. Features to be noted are the cross-banding of the fiber which results from the striations of its contained myofibrils being in register, the close-packed myofibrils some of which are spraying out at the ends (compare with Plate I, Fig. 4), the delicate sarcolemma which has been turned back at one end of the fiber, and the position of the nuclei immediately under the sarcolemma. (Adapted, with kind permission, from a figure in Sir Wilfrid Le Gros Clark's "Tissues of the Body.")

bundles. By using microelectrodes, however, Barer (1948) was able to produce contraction of some but not all of the myofibrils within a single fiber, clear evidence of their existence as independent contractile elements. In other experiments, Barer was able to produce asynchronous contraction of myofibrils, an occurrence which naturally poses the question of what mechanism ensures the normal synchronous contraction of all the myofibrils in a fiber. As noted earlier, the sarcoplasmic reticulum may provide the answer.

Myofibrils are elongated threadlike structures which measure about 1 μ or less in thickness; small as that may seem the electron microscope has revealed each to be packed solidly with still smaller elements called myofilaments.

Just as the muscle fiber shows alternate light and dark bands, so does each myofibril, and it would appear that the appearance of cross striation presented by the whole fiber results from the fact that the light and dark bands of its component myofibrils are in register with one another; that is to say they lie in approximately the same transverse plane (Text Fig. 3).

Since the myofibrils are the contractile units of muscle, their structure must be considered in detail, and since a wealth of terms has been employed in the past to describe their structure some pains will be taken with nomenclature. For further details of the multitude



TEXT FIG. 4. Diagram of the main striations seen in a single myofibril (rabbit). This figure is adapted from Barer (1948) whose illustration was based on the electron microscope photographs of Hall *et al.* (1946). The lettering indicates the named bands. The extent of one sarcomere is from one Z band to the next. Compare with Plate I, Fig. 2.

of terms which has been applied to them, Jordan (1933) and Honcke (1947) should be consulted. In text Fig. 4, the named portions of one myofibril are shown. As mentioned earlier, each myofibril when viewed with the light microscope consists of alternate dark and light portions. When examined by polarized light, however, the dark portions are clearly birefringent and are therefore called anisotropic bands, or in short the A bands. They are also frequently referred to as Q bands, from the German "querscheibe" meaning transverse disc. The light portions, often said to be non-birefringent, are in fact weakly birefringent, but the difference is sufficiently marked to merit their being called isotropic or in short the I bands; they are also called J discs (the German equivalent); further alternative names are clear discs, light bands, and hyaline substance. Other than the main A and I bands the only other striation which can be recognized with any certainty in the living fiber is the Z disc. This appears as a narrow dark line in the middle of the I band and was formerly thought to be evidence of a membrane called Krause's membrane.

The length of myofibril bounded by two Z bands is called a sarco-

ere, and is regarded as a structural unit within which certain ıanges are postulated to occur during contraction[1]. The designation band comes from "zwischenscheibe" which means intervening disc; :her names by which this feature of the myofibril has been known 'e telophragma, Dobie's line, end disc, and, alas, several more.

Additional features of the myofibril which may be detected from ı examination of fixed and stained material will now be described, ıd it is of interest to note that the detailed observations made by the .oneer histologists in this field have been confirmed by present day orkers using the electron microscope. It was known long ago that ıe appearances presented by the myofibrils are variable (Bowman, 340). It can now be stated that none of the cross bands is constantly resent during all phases of contraction and relaxation, or phases of retch (Bennett and Porter, 1953).

Within the A band there may be recognized a lighter region which known as Hensen's disc or the H band—"heller" meaning clearer : brighter. Its presence signifies a stage in contraction and it does not ppear in the fully relaxed fibril; however in a fibril which is stretched may be seen (Jordan, 1933). Bisecting the A band the M band may e seen; this is also known as the "mittelscheibe", i.e. intermediate isc, mesophragma, or median membrane.

About midway between the Z and A bands, there is less frequently bserved a narrow dark striation known as the N band or disc (somemes called the accessory disc of Engelmann), so that each I band will ıen show three striations, a central Z band flanked by an N band on ıch side. Since the Z bands are taken as limiting the sarcomeres, ıch sarcomere is sometimes described as possessing a terminal disc t each end between the N and Z bands. This may be called the E isc of Merkel. The name N derives from "nebenscheibe", "neben" ıeaning next to or beside.

Detailed consideration of the significance of these various structures ı the myofibril nowadays soon leads the microscopist into the field f protein chemistry, as a later chapter will show. No mention has yet een made concerning the measurement of the various bands decribed. If it were, in fact, the case that the different bands are sharply lelineated, some figures might well be expected, and measurements f band widths do appear in the literature. However, the findings of Bennett and Porter (1953) would seem to urge the greatest caution in

[1] But see Huxley, A F (1957). Progress in Biophysics, 7, 255

accepting precise assessments of band width. As Bennett (1955) points out, his recent work with Porter using the electron microscope has revealed that what had been observed earlier with the polarized microscope is quite true, viz. that there is a gradual transition between the A and I bands, and that a decision as to where one band stops and the other starts is purely arbitrary. It would seem wise to accept the advice given by Bennett and Porter when they stress that in defining band limits the basis of interpretation should be densitometric tracings of micrographs, whether obtained with the light or with the electron miscroscope[2].

8. *Organelles and Inclusions in the Striped Muscle Fiber*

Thus far it has been noted that the striated muscle fiber consists of a thin membrane, the sarcolemma, within which are nuclei, sarcoplasm, and myofibrils. The sarcoplasm is simply the cytoplasm of the muscle fiber within which, as in cells elsewhere, two kinds of formed elements can be recognized, organelles and inclusions. The name organelle is given to those bodies which are structurally specialized parts of the cytoplasm and could therefore be regarded as part of the living substance of the cell; whereas the term inclusion embraces the various particles of nonliving material which may be present. As Bloom (1957) has pointed out, however, advancing knowledge of the chemistry and submicroscopic structure of protoplasm makes the complete separation of cellular constituents into living and nonliving appear less apposite than formerly.

Of the organelles within striped muscle fibers, the myofibrils and sarcoplasmic reticulum have been discussed. In suitable preparations, a small Golgi apparatus can also be frequently seen at each pole of the nucleus. Mitochondria, sometimes called sarcosomes, are also present and were successfully demonstrated by the light microscope more than seventy years ago by Retzius. The use of the electron microscope in recent years has added greatly to our knowledge of these small but important structures (Bennett, 1955; Harman, 1955). Bennett found that in both number and arrangement they varied from muscle to muscle; thus in the flight muscles of the fowl mitochondria are scanty and irregularly disposed, whereas in the gracilis muscle of the mouse they are abundant. In this muscle, they are most common in the sarcoplasm just opposite the I band and straddling the Z band, orientated with their long axis transverse so as partially

[2] The recent paper by Huxley, H. E. (1957) has clarified much that was hitherto obscure on the relationships between the A and I bands.

to surround the myofibrils. Other sites where mitochondria are found are along the A bands where they are disposed longitudinally, the paranuclear sarcoplasm just off the poles of the nuclei, and in the subsarcolemmal sarcoplasm where dense masses may be present between the sarcolemma and the myofibrils. In the sartorius muscle of the frog, Bennett found dense masses of mitochondria arranged in longitudinal strands between the myofibrils. In the flight muscles of insects, they are also large and numerous.

Harman (1955) describes two types of granule in muscle, mitochondria similar to those of other tissues, and sarcosomes which are peculiar to muscle. In a study of pigeon breast muscle, Harman found the two particle types to show a close chemical similarity in regard to phospholipid, phosphoprotein, and nucleic acid content, but in their morphology many points of difference could be demonstrated. His paper should be consulted for details.

Inclusions which may be present in striped muscle fibers include glycogen granules of irregular size and shape, situated in the sarcoplasm near the junctions of the A and I bands of the myofibrils, and fat granules which will be further mentioned when red muscle is discussed.

D. Hypertrophy of Voluntary Muscle

The capacity of voluntary muscle to increase in size with exercise is common knowledge, and it would appear that such hypertrophy results not from an increase in the number of fibers present in the muscle but from enlargement of the diameter of the individual fibers due to the formation of more sarcoplasm. The highly specialized nature of striated muscle fibers when fully formed is in harmony with the belief that they probably do not undergo proliferation by cell division, and indeed there is evidence that their number does not increase in the human embryo after it has reached the age of four or five months, when it is 17 cm. in length (MacCallum, 1898).

E. The Blood Supply of Voluntary Muscle

Voluntary muscle has a rich blood supply derived from branches of neighboring arteries. The vessels enter with the nerves along a line which is frequently definite enough to receive the name of neurovascular hilum (Brash, 1955). From the epimysium the arteries travel into the substance of the muscle along the strands of perimysium,

dividing as they do so, the various branches of the entering vessels establishing free anastomoses with one another. Curiously, these anastomoses which show up well in injected material, do not function very efficiently should one of the supply vessels be ligated in an experimental animal (Clark and Blomfield, 1945). The finer branches of supply come to lie transversely to the long axes of the muscle fibers and from them arise the capillaries which course between the fibers, lying in the endomysium. It is noteworthy that the arteries and veins run together up to the point at which the terminal arterioles and venules are given off, but thereafter these small vessels arise alternately; this arrangement presumably allows the intervening capillary to run in a relatively direct course from arteriole to venule and so



TEXT FIG. 5. Drawing of injected capillaries in a teased portion of voluntary muscle. Slight focussing was required during drawing as all the vessels shown did not lie in exactly the same plane.

expedites rapid removal of metabolites (Clark, 1952). For the most part, capillaries run longitudinally between the individual muscle fibers. With their frequent linkage by transverse vessels, which pass over or under the intervening fibers, a fine capillary network with narrow oblong meshes is formed (Text Fig. 5).

In the immediate neighborhood of motor end-plates the capillary anastomoses are especially well developed (Wilkinson, 1929), an arrangement in harmony with the conception of these sites being particularly active metabolically. In the red muscles of the rabbit, Ranvier (1874) stated that the capillaries run a more tortuous course than in white muscle and also that the cross connecting vessels frequently show considerable dilatations. These enlargements have been considered to act as reservoirs for oxygen from which the muscle fibers can be supplied during sustained muscular contraction, at a time when the capillary circulation is impeded.

Lymphatic vessels are present in the epimysium and perimysium but seem to be absent from the endomysium between the individual fibers. If this is true, and Aagaard (1913) seems to be alone in describing a lymphatic capillary plexus around the fibers of voluntary muscle, then voluntary muscle might be said not to possess lymphatic vessels.

F. Red and White Muscle

It is common knowledge that muscle color is not uniform throughout the animal kingdom or indeed in the muscles of the same animal, and the white breast muscle and pigmented leg muscle of the domestic hen are familiar to all.

In a few mammals, such as the rabbit and guinea pig, red and white muscles occur separately, but in most the muscles are a mixture of red and white fibers. It is now fully established that the redness of a fiber depends upon its content of muscle hemoglobin, or myoglobin (Needham, 1926; Millikan, 1939; Biorck, 1949), and the description of a fiber as red should signify this and not certain microscopic appearances shortly to be mentioned.

The brief statements on red and white muscle which appear in the standard textbooks are based on the observations made by Ranvier (1873, 1874). In the rabbit he investigated the semitendinosus (red) and the adductor magnus (white) muscles and concluded as follows: red muscle fibers are more distinctly striated longitudinally but their transverse striation is less regular; red fibers have a larger amount of sarcoplasm and more nuclei than white fibers; and their nuclei occur within the depths of the fibers as well as beneath the sarcolemma. The features of the capillary blood vessels of red muscle as described by Ranvier have been mentioned earlier. Concerning the response of the two types of fiber to stimulation, Ranvier showed that red fibers have a slower, more prolonged contraction.

Although before Ranvier's day it had been shown that the redness of muscle was due to its content of muscle hemoglobin and not to richness of blood supply, and also that the fibers of the rabbit's red muscles were more granular than those of its white muscles, he was the first to provide evidence of a correlation between the contraction speed of a fiber and its histological appearance and pigment content. Admittedly, Lankester (1871) had suggested an association between red pigmentation and prolonged activity, but Ranvier's experimental evidence was the first of its kind.

Later studies have not left the situation so clear-cut; not only are there conflicting opinions regarding the association of speed of contraction with the histological features of muscle fibers but the actual histological appearances have been misinterpreted. Thus it is known that there are features such as granule content and sarcoplasm content which affect the appearance of muscle fibers cut in cross section; when there are many granules present in a fiber it appears dark in section, while another fiber which contains few granules appears light. Now it is quite true that many red muscles commonly show a high percentage of dark fibers, but their redness does not depend on this character for white muscle may have fibers which appear just as granular as those of red (Bullard, 1912).

It is also true that the fibers of red muscle on the whole contain more sarcoplasm than those of white, but again this is not always so; fibers of red muscle may contain little sarcoplasm, and those of white may contain much.

Further, the other structural features such as fiber size and nuclear arrangement cannot be taken as diagnostic of red or white muscle. In a recent study of the human gastrocnemius and soleus muscles, which are usually stated to contain a high proportion of pale (white) and red fibers, respectively, it was found that although the soleus contained more granular fibers than the gastrocnemius, there was no consistent difference in the myoglobin content of the two muscles. Nor did the position of the nuclei help in distinguishing the muscles (Walls, 1953).

Finally, with regard to speed of contraction it may be said that Denny-Brown (1929) believed that the red pigmentation does not appear to be essential to the slow type of contraction and is probably "the outward sign of some function not closely related to contraction."

It would appear that there are still problems to be solved concerning the significance of muscle color, and it may be that the answers will be provided by our pharmacological and physiological colleagues.

G. Tendons and Tendon Sheaths

The relation of tendons to muscle architecture and the advantages afforded by their presence having been discussed in Volume I, Chapter 1, their microscopic structure will be considered in this chapter. Tendons consist almost solely of white fibrous tissue, the collagen fibers of which form closely packed parallel bundles. The bundles do not remain

separate throughout their extent, for fibers are given off to, and received from, neighboring bundles so that successive cross sections of a tendon show slightly differing appearances. The surface of a tendon has a characteristic white, shining aspect; closer inspection, however, will often reveal light and dark streaks across its length, an appearance resembling that of watered silk [Schafer (1912)], which results from the somewhat wavy course taken by the collagen fibers when not under tension.

Surrounding the tendon, there is a sheath of dense areolar tissue from which septa carrying the blood vessels, nerves, and lymphatics pass between the secondary bundles into which the collagen fibers are grouped. The cellular component of tendon consists of fibroblasts, sometimes called tendon cells, arranged in single rows between the bundles of collagen fibers. In transverse section the cells appear stellate since the middle of each cell containing the nucleus occupies the angular space between several bundles of fibers, while lamellar extensions proceed from the cells into the bundle interspaces. The processes of the tendon cells may be considered to mark off, indefinitely it is true, the primary collagen fiber bundles. For the most part, tendon cells are not connected with one another, but it is sometimes possible to follow a lateral extension of a cell in one row into continuity with a cell of another row. When seen in surface view the tendon cells may appear rectangular, rhomboidal, or irregular, but seen in profile as in a longitudinal section of tendon, they appear as rods owing to their thinness. Elastic fibers are present to some extent in the connective tissue septa but otherwise tendon has very little elastic tissue.

In those parts of the body where tendons lie close to bone, friction is reduced by the presence of tendon sheaths. Essentially each is a closed tubular sac into which the tendon is invaginated, so that it is closely invested by one layer of the sheath which indeed follows it in its movements. The other layer of the sheath lines the thickened fibrous tissue which acts as a retaining band for the tendon. Within the tubular sheath, there is a little mucinous fluid, similar to the synovial fluid of joints, by means of which movements are facilitated. Not all tendons have well formed sheaths, and around those which lack them it will be found that there is loose connective tissue, the meshes of which contain the same kind of mucinous fluid.

Composed as it is predominantly of intercellular material, the

metabolic activity of adult tendon is low and its blood supply correspondingly meager (Edwards, 1946). Blood vessels enter tendon both at its muscle end and at its periosteal end, and in the case of some tendons with sheaths, along reflections of the sheaths as well. Such reflections are called mesotendons. But, threefold though its blood supply may be, tendon is poorly vascularized. During development, tendon has quite a good blood supply, but with advancing age this becomes greatly reduced.

H. The Muscle-Tendon Junction

The precise manner in which the force of the contracting myofibrils is transmitted to the muscle tendon has been a matter of controversy for years. Two main theories have had their supporters: the first, or continuity theory, supposes that there is direct union of the myofibrils and the tendon fibrils, while according to the second, or sarcolemma theory, there is no such continuity and the sarcolemma forms a limiting membrane between the substance of the muscle fiber and the tendon elements. Long (1947) and Barer (1948) review the literature on this matter. Long, who studied the development of the muscle-tendon attachment in the rat, found no evidence in support of continuity. In both fetal and adult stages, the muscle fibers are enclosed by a limiting membrane both at their terminations and along their sides and the myofibrillae end on the internal aspect of this membrane. In the attachment region, the reticular fibers which envelop the ends of the muscle fibers continue proximally as the sarcolemmal and endomysial reticulum, and distally they are continuous with the tendon fibers.

However, Barer does not feel that the protagonists of either view can prove their case by using conventional fixed and stained material, but rather that the observation of whole muscle fibers holds out the best means of a solution. In his studies of living muscle fibers, including microdissection, he obtained no evidence of any special connection between myofibrils and tendon fibrils. Thus, when retraction clots form in the junctional zone, a clear area of sarcolemma is left between the clot and the tendon fibrils, an occurrence which also follows spontaneous herniation of the muscle substance after treatment with dilute acids. From microdissection of such material, Barer believes that the sarcolemma is continuous over the end of the fiber and that the tendon fibrils are attached to the outside of the sheath.

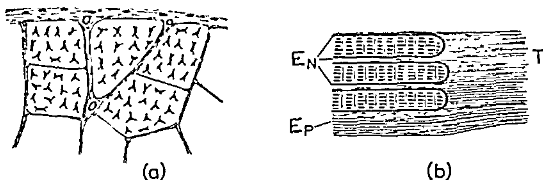
The idea that there is any structural continuity between the muscle substance and the sarcolemma is not supported by the facts. The statement has been made that the Z bands are really rigid membranes which stretch right across the fiber, linking the Z bands of neighboring myofibrils, and are circumferentially attached to the sarcolemma. This would, if true, offer a means for the transmission of the contractile force to the sarcolemma, and beyond it to the endomysial connective tissue and so to the tendon. But the evidence against such Z band-sarcolemmal continuity is overwhelming. As was noted in an earlier section, the sarcolemma can be made to separate from the muscle fiber substance in a great many ways, and Bowman himself showed that the whole myofibrillar and sarcoplasmic content of a fiber could be expressed without difficulty through the sarcolemma. There is obviously no firm bond between sarcolemma and the contractile elements. In any event, the Z bands are myofibril bands, and many features have been observed which dispute the notion of their being united uniformly across the fiber to form a continuous membrane. For example: columns of ice can advance between bundles of myofibrils without hindrance; nuclei have been seen to move between myofibrils without seemingly being hindered by cross membranes; oil droplets may be pushed along inside a muscle fiber with no difficulty; and finally there is the time-honored observation of Kuhne (1863), who observed a tiny living parasite—a nematode—inside a fresh muscle fiber, moving freely up and down inside the fiber without permanently affecting the appearance of the cross-striations. (Actually, for reasons which he gives in full, Speidel (1939) thinks too much has been made of this)

Barer believes it possible to explain the transmission of contractile force from myofibrils to sarcolemma by a frictional or viscous relationship between these structures; a viscous gel when contracting would exert a pull on its surrounding membrane and thence to the endomysial connective tissue

Bennett (1955) gives a brief account of such electron microscopic observations as have been made on the junctional zone between muscle and tendon. Simply put, the evidence points to a direct transmission of tension at the ends of the myofibrils. The myofibrils stop a short way from the sarcolemma, and tapering strands of fibers of unknown nature continue through the intervening sarcoplasm space, appearing as noncontractile continuations of the myofibrils; inserting

on the sarcoplasm opposite the attachments of these connecting fibrils are the tendon fibrils which therefore form an extension or extrapolation of them.

With regard to the Z bands, Bennett states that their dense material is confined to the myofibrils and does not extend across the sarcoplasm; such material as does extend from the Z band of one myofibril to that adjacent to it is weak and easily stretched, and moreover sarcoplasm of varying thickness intervenes between the sarcolemma and the myofibrils. Clearly any view of contraction which invokes the attachment of complete Z membranes to the sarcolemma at the periphery of the fiber is untenable. Text Fig. 6 is a diagrammatic representation of the muscle-tendon junction and of the structure of tendon.



TEXT FIG. 6. Diagrams of (a) the structure of part of a tendon in cross section, and (b) part of a muscle-tendon junction. In the cross section note the stellate appearance of the tendon cells whose extensions faintly demarcate areas occupied by the primary bundles of collagen fibers (not represented). The secondary bundles are surrounded by septa of fibrous tissue which penetrate the tendon from its investing connective tissue sheath. Blood vessels are present in the sheath and in the septa, but elsewhere are sparse. At the muscle-tendon junction some of the connective tissue fibrils of the tendon (T) become attached to the sarcolemma of each muscle fiber; others become continuous with the connective tissue of the epimysium (Ep), perimysium (not represented), and endomysium (En).

II. SMOOTH MUSCLE

A. DISTRIBUTION

Smooth muscle is known also as unstriped, unstriated, involuntary, plain, or visceral muscle. This tissue has a widespread distribution in the mammalian body and subserves its vegetative functions. Whereas the somatic or striped musculature adjusts the organism to its external environment, plain muscle may be said to fulfill the role of maintaining the internal environment or physiological balance of the

body. Thus it is found primarily forming the contractile walls of the hollow viscera and of the blood vessels and large lymphatic vessels. In the alimentary canal, it is present in man from about the middle third of the esophagus to the anal canal, and by its activity ensures that the ingested food is carried along its length and thoroughly mixed with the digestive juices, and finally that the residue is excreted. In the respiratory system, it occurs from the trachea above to the alveolar ducts below. The complement of smooth muscle in the walls of the urinary and genital tracts of both sexes ensures on the one hand the excretion of urine and on the other the survival of the race. Other sites of its occurrence are the dermis of the skin where it forms the arrectores pilorum (the little muscles which pull on the hair follicles); the subcutaneous tissue of the scrotum, penis (glans and prepuce), of the perineum, of the areola of the nipple, and of the nipple itself; the eyeball, in which it forms the musculature of the iris and ciliary body; the capsule and trabeculae of the spleen in which the visceral muscle component is much less in man than in the pig, dog, or cat, and less than in the ox or sheep; and finally it may be mentioned that smooth muscle also occurs in the walls of the larger ducts of glands.

Like the striped and cardiac variety, smooth muscle never shows direct contact of its specific structural elements as is seen, for example, in epithelial tissue. Always there is combined with the contractile elements an amount of connective tissue, and although this may be present between the muscle fibers in very small quantity indeed, it is always there.

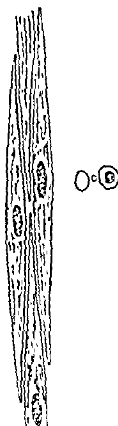
In some situations, notably the skin but elsewhere too, for example, the villi of the small intestine, smooth muscle fibers may be observed in quite small groups, or even singly, surrounded by connective tissue; but for the most part smooth muscle is found disposed in sheets or layers in which the individual fibers are set very close to one another. The arrangement of muscle fibers into discrete fasciculi, which is so characteristic of voluntary muscle, is best exemplified in smooth muscle by the arrectores pilorum.

B. The Muscle Fibers of Smooth Muscle

1. *Size and Shape*

As has been noted, the study of fresh voluntary muscle fibers teased apart from one another in, for example, Ringer's fluid, has provided useful information. Such a procedure using smooth muscle is not

successful, and indeed it is very difficult to view fresh smooth muscle fibers satisfactorily. Even in histological preparations, the outlines of the muscle fibers are not always well defined, and it may be a matter of difficulty to observe with precision the boundary between the fiber and its surrounding connective tissue. The use of special stains such as those of Van Gieson which colors collagen fibers red and smooth muscle yellow, or Mallory which yields blue and red, respectively,



TEXT FIG. 7. Diagram of the structure of visceral muscle fibers. Points to be noted are their spindle form with long tapering ends, the central nucleus occupying the widest part of each fiber, and the varying sizes of the fibers when seen in cross section. Compare with Plate II, Figs. 1, 6, and 8.

helps not only in observing the structure of the muscle fibers but also in avoiding the mistake of confusing smooth muscle with collagen fibers.

The muscle cells or fibers are simple contractile elements, usually in the form of elongated spindles with sharply pointed ends which in a few cases may be bifurcated. The thickest part of each fiber lies about the middle of its length and is occupied by a single elongated nucleus (Text Fig. 7). All smooth muscle fibers are not of the same length,

but while there is considerable variation it is not to be compared with that found in striped muscle. Most fibers are between 50 and 250 μ in length, with a greatest diameter of between 5 and 10 μ, but shorter and longer fibers occur. In the pregnant human uterus, smooth muscle fibers may reach a length of 500 μ, while in the walls of blood vessels the fibers may be as short as 20 μ; such short fibers are usually relatively thick, with nuclei of short ellipsoidal form. The smallest fibers, such as those found in blood vessels, may be rather irregular in shape, short, broad, and usually flattened, with their ends deeply forked or even more extensively branched. Some of them could well be described as star-shaped.

2. *Myo-epithelium*

For the sake of completeness, it might be proper at this point to mention a type of cell which is found in association with the secretory elements of the mammary glands, sweat glands, lacrimal glands, and the glands of the oral cavity. These are known as myo-epithelial, basal, or basket cells, and they are able to act like smooth muscle cells and so aid movement of the secretion into the excretory ducts. They are situated between the secretory cells and the basement membrane, and are either spindle-shaped or branched with cylindrical nuclei. They can be well seen if Masson staining is employed and they then appear bright red. Unlike smooth muscle cells, myo-epithelial cells are derived from ectoderm, not mesenchyme, and doubt has been expressed as to whether they are true unstriped muscle cells. The same difficulty attaches to the nature of the musculature of the iris, which differentiates from the ectoderm of the optic cup, and it has been suggested that the iridial muscles are really myoneural elements rather than true smooth muscle cells (Vinnikow, 1938).

3. *Myofibrils*

Within the sarcoplasm or more fluid part of the cytoplasm of smooth muscle fibers, there are present fine, longitudinal threads which may be made evident by treating the tissue with macerating fluids such as nitric or trichloroacetic acid. Much less obvious and definite than the myofibrils of striated muscle, they have not yet been identified with certainty in fresh tissue. Often even in fixed and stained material they cannot be recognized, and the cytoplasm appears homogeneous. As seen with the light microscope, the myofibrils, which might well be

called myo-filaments, appear as structureless fibrils something less than 1 μ in thickness, running the length of the cell. They are anisotropic in their whole extent, quite lacking the cross banding seen in striated muscle myofibrils, at any rate in vertebrates. Sometimes a smooth muscle fiber shows a few irregular cross bands which are considered to be contraction bands persisting with the death of the cell. There is a large variation in the number and thickness of the fibrils from one smooth muscle organ to another, but as a rule the boundary fibrils are coarser and stain more deeply than those more centrally placed (Fischer, 1914). In many rodents, the smooth muscle cells contain fibrils which are few in number but unusually stout. Sobotta (1930) suggests they may be tonofibrils. It might be thought that in transverse section all smooth muscle fibers would show dots corresponding to the myofibrils, but in practice it frequently happens that only a proportion do.

It is generally agreed that with visible light smooth muscle fibers lack a sarcolemma, but on the question of whether or not the fibers are anatomically separate structures, there is as yet no final agreement. In invertebrates, it is not uncommon to find smooth muscle fibers forming true syncytial connections with others, but although cell bridges and fibrillar continuity have been described in vertebrates the balance of evidence is against such an arrangement. Roskin (1936) appears to favor an intermediate view. This author considers that while the smooth muscle fiber does not in most cases represent a unit, neither does the fibrillar network show all the characteristics of a syncytium; he suggests the name "myon" for a single strand of smooth myofibrils which through anastomoses can form "myons" of higher order. However, it must be repeated that most workers at the present time do not consider smooth muscle to be syncytial in character. Haggqvist (1956) should be consulted for details.

4. *Nuclei*

In the usual arrangement of smooth muscle in sheets, the fibers are so disposed that the thickest part of one fiber lies alongside the tapering ends of its neighbors (an exception is found in the small muscles of the hairs, in which the nuclei lie side by side, producing a localized thickening of the muscle). It follows that in a cross section of smooth muscle, relatively few nuclei are seen; some of the fibers are of course cut through their widest parts and so show nuclei, but the remainder are

cut elsewhere and, accordingly, fiber sections of all sizes and of irregularly round or prismatic shape are present. The very smallest sections are those of the pointed ends of the fibers.

Each smooth muscle fiber has a single elongated nucleus which occupies its thickest part. This nucleus commonly is seen in a slightly eccentric position when seen in cross section. The length of the nucleus varies with that of the fiber, but not to the same extent, and its average dimensions would be about 15–20 μ by 2–3 μ. The nucleus is elongated in the long axis of the fiber and has pointed or rounded ends, but owing to contraction of their containing muscle fibers, nuclei are frequently seen in stained preparations to be contorted to a greater or lesser degree. Spiral twisting is very common (Plate II, Fig. 6). In very short fibers, the nuclei have the form of short ellipsoids. Within the nucleus, there are two or more nucleoli and a net of chromatin which does not stain very densely. Adjacent to the nucleus, in a small depression in its membrane, there is a diplosome but no attraction sphere.

At the ends of the nucleus, the sarcoplasm has a tendency to accumulate to some extent, and small lipid granules may be present within it in these places. Other intracellular inclusions are glycogen granules, mitochondria, and a Golgi apparatus. Unless separately stained for, the glycogen granules will be lost and their former sites will be indicated by empty spaces.

C. Connective Tissue of Smooth Muscle

The manner by which the individual fibers of smooth muscle are bound together must now be considered. In many preparations, the impression first gained is that the fibers are in direct contact with their neighbors, but suitable staining shows this not to be so. Connective tissue in the form of delicate non-nucleated strands of reticular fibers surrounds each fiber. The name membranellae has been given to this fine tissue which is responsible for the difficulty experienced in attempting to tease the muscle fibers apart. Preliminary treatment with an agent such as caustic potash dissolves the membranellae and so enables the muscle fibers to be isolated. The reticular fibers, which can be considered to form intimate sheaths for the muscle fibers supporting them and binding them together, are continuous with the stouter strands of connective tissue which separate the layers or bundles of smooth muscle fibers This connective tissue contains collagen and

elastic fibers, fibroblasts, and wandering cells, and also serves to conduct the blood vessels and nerves. The muscles which are attached to hairs exert their pull through networks of elastic fibers which correspond to the tendinous insertions of skeletal muscles. This close association of smooth muscle with elastic fibers is in fact so general throughout the body that the term *myoelastic tissue* is sometimes applied to it.

It has been noted that the cytoplasm of smooth muscle cells consists of myofibrils surrounded by sarcoplasm, and that the former are not always to be seen in every section examined. It follows that difficulty may be experienced in telling visceral muscle from collagenous connective tissue; it is a help to remember that of the two fiber types, visceral muscle fibers appear more darkly stained with acid aniline dyes such as eosin.

The blood supply of smooth muscle is sparser than that of skeletal muscle, but as in that tissue the capillaries run longitudinally between the fibers.

D. Hypertrophy of Smooth Muscle

The spectacular increase in size and weight of the uterus during pregnancy—from an organ some 8 cm. or less in length and weighing about 30 g. (human), to one of 32 cm. length and 1000 g. weight—represents the physiological response of its tissues, including the smooth muscle component. But that smooth muscle may be stimulated to increase in bulk by less happy circumstances is witnessed all too frequently by the thickened and tortuous arteries of the hypertensive patient. With regard to the means whereby smooth muscle increases in bulk, i.e. hypertrophies, there are clearly three possibilities: first, the individual cells may increase in size. That this does happen is undoubted, and according to Lange (1939), it is the enlargement of the individual fibers rather than an increase in their number, which accounts for almost all the hypertrophy shown by smooth muscle. Second, the muscle fibers may increase in number, i.e. proliferate, by mitotic division. This also is known to happen (Muir, 1929) but the degree to which it occurs is limited. Whether smooth muscle fibers are to be regarded as "highly specialized" (Clark, 1952) or "not very highly differentiated" (Ham, 1957) seems to be a matter of opinion, but whichever view is accepted it appears that these cells can divide by mitosis. Third, it is possible that new smooth muscle cells may arise

by differentiation of undifferentiated mesenchymal cells which persist in many parts of the body into adult life, e.g., the undifferentiated perivascular cells found alongside capillaries. It seems likely that such cellular transformation does occur in the adult but absolute proof of this is difficult to obtain.

III. CARDIAC MUSCLE

A. General Structure

Cardiac muscle tissue is found only in the heart and surrounding the mouths of the great veins which enter it. In some ways it resembles both voluntary and smooth muscle, yet in its rhythmic, unceasing activity from early embryonic life until death, it stands alone. Like voluntary muscle, its contractile elements show transverse striation; like visceral muscle, its fibers possess centrally placed nuclei, and further, it too has an autonomic innervation.

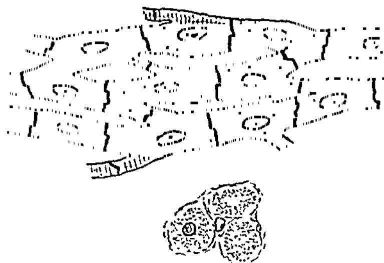
The composition of its fibers is essentially similar to that of voluntary muscle. It has the same basic constituents—sarcoplasm, myofibrils, nuclei, and sarcolemma, but in their form and caliber the fibers show distinct differences. In transverse section, they are less regular in shape. Their most characteristic feature is that in longitudinal section they can be seen to give off branches at acute angles and so effect anastomoses with adjacent fibers. The branches are more slender than the parent fibers which themselves seldom measure more than 15 μ in cross section; as will later be mentioned this size may be greatly exceeded by those fibers of heart muscle which are believed to be specialized for the purpose of conducting the impulse for cardiac contraction. The branching nature of cardiac muscle fibers has in the past led to the belief that the whole heart musculature in fact formed a syncytium, but recent findings made with the electron microscope and shortly to be discussed have brought about a change of view.

The question of whether or not cardiac muscle fibers possess a sarcolemma has been much debated. The matter has been well reviewed by Cohn (1932) It may be said that a sarcolemma does exist but it is much finer and more difficult to demonstrate than in voluntary muscle.

The sarcoplasm of cardiac muscle fibers is not only very abundant but also distinctly granular, and because of this the transverse striation of the fibers is less distinct than that of voluntary muscle; the longi-

tudinal striation however is very evident. Embedded in its sarcoplasm are myofibrils which are quite similar to those of voluntary muscle and show the same alternate sequence of light and dark bands which confers the cross-striated appearance upon the fibers as a whole. The A, I, and Z bands can all be recognized. As in voluntary muscle, the sarcoplasm contains mitochondria, fat droplets, and glycogen granules.

The nuclei, which are rather pale, occupy the middle of the fibers, in contrast to the sub-sarcolemmal position favored in voluntary muscle. In shape they are somewhat ellipsoidal, but it is not uncommon



TEXT FIG. 8. Diagram of the structure of cardiac muscle in longitudinal and transverse section. Points to be noted are the branching of the fibers which are striated both longitudinally and transversely, the centrally placed nuclei, and the intercalated discs. Of the three fibers shown sectioned transversely, one is cut through the nucleus, one through the polar cone of myofibril-free sarcoplasm, and one between this zone and a disc. Compare with Plate II, Figs. 3, 4, 5, and 7.

to see nuclei with square-cut ends. At each end of the nucleus there is a cone-shaped accumulation of sarcoplasm free from myofibrils, the polar cone, in which are present small yellowish-brown pigment granules which increase with age. In the condition of "brown atrophy," this pigment becomes very obvious within the shrunken cardiac muscle fibers.

B. INTERCALATED DISCS

Separating the cardiac muscle fibers into segments some 50–120 μ in length, each of which usually contains one nucleus, seldom two, there are transverse bands less than a sarcomere in width known as intercalated discs. Those frequently traverse the fiber in a *series of* short steps (Text Fig. 8). They increase in number with age and are in

by differentiation of undifferentiated mesenchymal cells which persist in many parts of the body into adult life, e.g., the undifferentiated perivascular cells found alongside capillaries. It seems likely that such cellular transformation does occur in the adult but absolute proof of this is difficult to obtain.

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fact late in developing, for Cohn (1909) failed to find them in fetal hearts or in the hearts of children. In the hearts of other animals, however, they appear earlier and they have been found by Witte (1919) in fetal pigs of the 76-mm. stage, and by Jordan and Banks (1917) in the beef heart at the second to the third month. Jordan and Steele (1912) observed them in the guinea pig during the last week of gestation, and also in the heart of a four-year-old child.

Many views have been advanced with regard to the significance of the discs and even yet the matter has not been settled. The idea that they represent cell membranes goes back almost a century and indeed there are features of cardiac muscle that support this belief. Thus they show up well after treatment with silver salts; the segments which they demarcate commonly contain one nucleus; fragmentation of the fibers, induced by maceration or as the result of pathological myocardial segmentation, occurs at the discs; they increase in number and complexity with growth and activity of the heart; there may be a different degree of contraction of the fiber on each side of a disc (Van Breeman, 1953); finally, the fact that the discs are attached to the sarcolemma would allow of the segments being regarded as individual cells bounded all round by a cell membrane.

Against the view that the discs are cell membranes are the facts of

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fibers cut longitudinally. Note the peripheral cross-striation in these large fibers. Compare with Fig. 3. Silver impregnation. Magnification: × 225.

FIG. 3. Ordinary ventricular myocardial fibers from part of the same section shown in Fig. 2. Note the smaller size of the fibers, their complete cross-striation, and their centrally placed, clear nuclei. Silver impregnation. Magnification: × 225.

FIG. 4. Longitudinal section of human heart muscle to show the branching fibers, their central nuclei many of which seem somewhat square-cut, and the vascular connective tissue between the fibers. Hematoxylin and eosin. Magnification: × 290.

FIG. 5. Longitudinal section of human heart muscle stained with Mallory's phosphotungstic acid and hematoxylin to show the intercalated discs. Magnification: × 680.

FIG. 6. Transverse section of human artery to show the contorted nuclei of the contracted smooth muscle fibers. Hematoxylin and eosin. Magnification: × 225.

FIG. 7. Longitudinal section of rabbit heart muscle to show apparent continuity of myofibrils through an intercalated disc. Recent electron microscope studies have shown this appearance to be illusory. Magnification: × 1140.

FIG. 8. Smooth muscle fibers cut in cross section. Fiber sections of very varied size are seen since some of the fibers have been cut through their widest parts and others through their tapering ends. For this reason, too, only some fibers show a nucleus. Illeum of dog Azan. Magnification: × 1250.

FIG. 9. Cross section of Purkinje fibers in the moderator band of ox. Ordinary ventricular fibers to the right. Silver impregnation. Magnification: × 225.



PLATE II

FIG. 1. Transverse section of the middle third of human esophagus to show smooth muscle fibers and voluntary muscle fibers cut longitudinally side by side. Hematoxylin and eosin. Magnification × 830.

FIG. 2. Longitudinal section of moderator band of ox heart to show Purkinje

response to functional requirement in these two vertebrate classes, and that it does in fact act as the conducting system, Davies and his co-workers have carried out extensive comparative and experimental studies which carry great conviction (Blair and Davies 1935; Davies, 1930; Davies and Francis, 1946; Davies *et al.*, 1956). Forty years ago, it was held that the well developed conducting system of the mammal represented a remnant of more extensive tissue of similar structure in lower vertebrate hearts, but Davies and his colleagues have found themselves unable to support this view in any way. Prakash, (1954) and earlier papers, and Bhatnagar (1957), however, dispute the neomorphism of the system in birds and mammals.

The specialized conducting tissue of the mammal, which is closely similar to that of the bird, will now be described.

2. *The Sinu-atrial Node*

First described by Keith and Flack (1907), this structure is now accepted as the site of initiation of the impulse for cardiac contraction, and may be called the pacemaker. It is of horseshoe shape and embraces the ventral aspect of the termination of the superior vena cava. It is much more extensive than usually described, extending through the entire thickness of the atrial wall from epicardium to endocardium and, moreover, the anterior limb of the horseshoe extends fully halfway down the sulcus terminalis. It was originally thought to be composed of tissue intermediate in structure between nerve and muscle, and, accordingly, was given the noncommittal name of nodal tissue. Its fibers, however, can be shown with silver impregnation to be specialized cardiac muscle fibers, completely cross striated, of fusiform shape and branched. Intermingled with the nodal fibers is a considerable quantity of connective tissue. In diameter, the nodal fibers are smaller than ordinary neighboring atrial fibers. Data given by Blair and Davies for a fourteen-year-old human heart are nodal 2–7 μ and atrial 5–11 μ. Duckworth (1952) has shown that there is a considerable increase in the connective tissue content of the node just after birth. The nodal fibers are in direct continuity with the myocardial fibers of the right atrium, and these in turn with those of the left atrium. Although reports have been made from time to time of a direct connection between the sinu-atrial and atrio-ventricular nodes, there seems to be no real evidence that such exists. The impulse therefore travels between the two nodes via ordinary heart muscle.

their late appearance in development and their numerical increase w age—unaccompanied, it is believed, by corresponding cell divis Moreover, on the basis of appearances as seen with the light microsc it has long been believed that the myofibrils are continuous through discs (Plate II, Fig. 7). However, electron microscope studies h shown that this appearance is illusory (Van Breeman, 1953).

Other views regarding the discs are those of Heidenhain, who c sidered them to represent sites of longitudinal growth, and of Jor (1933), who thought they could be irreversible contraction ba Evidence in support of this latter opinion has been obtained by Be *et al.* (1949). Further details may be found in Volume II, Chapter

C. Blood Supply and Lymphatic Drainage

Cardiac muscle has an abundant blood supply and as in volun muscle, the capillaries follow the general arrangement of the mu fibers. They share the slit-like spaces between the myocardial fi and their branches with the loose connective tissue which corresp to endomysium. Unlike voluntary muscle, lymphatic vessels are ple ful and form a network throughout the intermuscular connective tis

D. Hypertrophy of Cardiac Muscle

Accompanying conditions such as hypertension and certain valvu diseases of the heart, when it is called upon to work against increa resistance, the cardiac muscle responds by an increase in size. T hypertrophic response is believed to be due solely to an enlargemen the existing cardiac muscle fibers and not to any increase in th number. The structure of heart muscle makes an investigation o problem such as this very difficult, but the work of Karsner *et al.* (19 seems to have established that no new fibers are formed. It may also stated as collateral evidence that investigations into the regenerat capacity of heart muscle have shown that this is insignificant (Ha son, 1947, Walls, 1948).

E. The Conducting System of the Vertebrate Heart

1. *General Observations*

In the hearts of mammals and birds, there is present structura specialized tissue which is generally considered to conduct the impu for cardiac contraction. In support of the belief that this tissue i neomorphic development which has undergone parallel evolution

fibers in the hearts of dog, cow, and man. In all three hearts, it was found that three types of Purkinje fibers were present as follows:

Type 1 fibers have a large diameter, three to five times that of myocardial fibers, and have large spherical nuclei arranged in groups of 2–4 in the center of the fiber. Sarcoplasm is abundant and in consequence, the myofibrils which are arranged mainly at the periphery appear relatively sparse. This type of Purkinje fiber is common under the endocardium but seldom penetrates the walls. In many cases, myofibrillar continuity of these fibers with those of type 2 was seen.

Type 2 fibers have a diameter about twice that of ordinary myocardial fibers and have less sarcoplasm than Purkinje fibers of type 1. Their myofibrils tend to be peripheral, although many are central, while their large and spherical nuclei are singly arranged. They can be observed to join with ordinary myocardial fibers or with type 3 fibers, and the transition is abrupt.

Type 3 fibers are only slightly wider than ordinary myocardial fibers and can be called a transition type. They have singly arranged, large, spherical nuclei, and the continuity which they establish with ordinary ventricular fibers is abrupt.

References

Aagaard, O. C. (1913). *Anat. Hefte* **47**, 493.
Banus, M. G., and Zetlin, A. M. (1938). *J. Cellular Comp. Physiol.* **12**, 403.
Bardeen, C. R. (1903). *Anat. Anz.* **23**, 241.
Barer, R. (1947). *J. Anat.* **81**, 259.
Barer, R. (1948). *Biol. Revs. Cambridge Phil. Soc.* **23**. 159.
Beams, H. W., Evans, T. C., Janney, C. T., and Baker, W. W. (1949). *Anat. Record* **105**, 59.
Bennett, H. S. (1955). *Am. J. Phys. Med.* **34**, 46.
Bennett, H. S. (1956). *J. Biophys. Biochem. Cytol* **2**, part 3 (Suppl.), p. 171.
Bennett, H. S , and Porter, K. R. (1953). *Am. J. Anat.* **93**, 61.
Bhatnagar, S. P. (1957). *Indian J Med. Sci.* **11**, 1.
Biorck, G. (1949). *Acta Med. Scand.* **133**, Suppl. 226, p. 1.
Blair, D. M., and Davies, F. (1935). *J Anat.* **69**, 303.
Bloom, W. (1957). "Textbook of Histology" by A. A. Maximow and W. Bloom, 7th ed , p. 17. Saunders, Philadelphia, Pennsylvania.
Bowman, W. (1840). *Phil. Trans. Roy. Soc. London* **130**, 457.
Brash, J. C (1955). "Neurovascular Hila of Limb Muscles." Livingstone, Edinburgh and London.
Bucciante, L., and Luria, S. (1934) *Arch. ital. anat. embriol.* **33**, 110.
Buchthal, F. (1942). *Biol. Medd. Kbh.* **17**, 1. Quoted by Barer (1948).
Buchthal, F. and Knappeis, G. G. (1946). Personal communication cited by Honcke (1947).
Bullard, H. H. (1912). *Am. J. Anat.* **14**, 1.

3. *The Atrio-Ventricular Node*

First described by Tawara (1906) this structure, which is smaller than the sinu-atrial node, lies in the lower part of the atrial septum. Its specialized fibers are thus in continuity with the ordinary myocardial fibers of both atria. The nodal fibers, which show complete cross striation, differ from those of the S.A. node in being rather thicker, although more slender than the fibers of the ordinary myocardium, more cylindrical, and much more branched.

4. *The Atrio-Ventricular Bundle*

In direct continuity with the A.V. node is the A.V. bundle, a structure which can be dissected with ease in the ox but with considerably more difficulty in man (Walls, 1945). The bundle runs forward under the septal cusp of the tricuspid valve to divide over the fleshy part of the interventricular septum into right and left limbs. The right limb forms a compact bundle on the right side of the septum from which it usually passes into the moderator band and so across the ventricular cavity to the base of the anterior papillary muscle; arrived there it breaks up into a subendocardial network of Purkinje fibers. The left limb is in the form of a broad, flattened band which descends for some distance on the left side of the septum before dividing into branches which pass across the ventricular cavity via the "false" tendons to reach the papillary muscles on the opposite wall; once there they break up into a plexus of Purkinje fibers beneath the endocardium.

5. *Purkinje Fibers*

In many mammals, the A.V. bundle and its limbs consist of large Purkinje fibers first described by Purkinje in 1845. In the human heart, however, the fibers in the bundle and the proximal parts of its limbs are no larger than the ordinary fibers—indeed, they are slightly narrower—and it is about halfway down the septum before Purkinje fibers can be detected in the limbs of the bundle.

The special endocardial network observed by Purkinje in the ungulate heart was composed of large, pale fibers, often with two nuclei, in which striation was limited to the periphery of the fibers. This description still holds good (Plate II, Figs 2 and 9), but it has recently been amplified considerably by Kugler and Parkin (1956), who reinvestigated the problem of whether or not there was direct continuity between ventricular Purkinje fibers and ordinary myocardial

Lange, K. H. (1939). *Gegenbaurs Morphol. Jahrb.* **84**, 363.
Lankester, R. (1871). *Arch. ges. Physiol. Pflüger's* **4**, 315.
Lindhard, J. (1926). *In* "Physiological Papers," (R. Ege, H. C. Hagedorn, J. Lindhard, P. B. Rehberg, eds.) p. 188. Heinemann, London.
Lockhart, R. D., and Brandt, W. (1938). *J. Anat.* **72**, 470.
Long, M. E. (1947). *Am. J. Anat.* **81**, 159.
MacCallum, J. B. (1898). *Bull. Johns Hopkins Hosp.* **9**, 208.
McMeekan, C. P. (1941). *J. Agr. Sci.* **31**, 1.
Mayeda, R. (1890). *Z. Biol.* **27**, 119.
Meara, P. J. (1947). *Onderstepoort J. Vet. Sci. Animal Ind.* **21**, 329.
Millikan, G. A. (1939). *Physiol. Revs.* **19**, 503.
Muir, R. (1929). "Textbook of Pathology," p. 158. Arnold, London.
Needham, D. M. (1926). *Physiol. Revs.* **6**, 1.
Oshima, T. (1938). *Japan. J. Med. Sci. I.* **7**, 59.
Prakash, R. (1954). *Proc. Zool. Soc. Bengal.* **7**, 27.
Purkinje, J. E. von (1845). *Arch. Anat. u. Physiol.* **12**, 281.
Ramsay, R. W., and Street, S. (1940). *J. Cellular Comp. Physiol.* **15**, 11.
Ranvier, L. (1873). *Compt. rend.* **77**, 1030.
Ranvier, L. (1874). *Arch. Physiol. norm. pathol.* [2], **1**, 1.
Robertson, D. D., and Baker, D. D. (1933). *Missouri Univ. Agr. Research Bull.* **200**, 1. Quoted by Meara (1947).
Roskin, G. (1936). *Z. Zellforsch. u. mikroskop. Anat.* **24**, 585.
Schafer, E. A. (1912). *In* "Quain's Anatomy" (E. A. Schafer, J. Symington, T. H. Bryce, eds) 11th, ed , vol. 2, part. 1, pp. 111, 175. Longmans, Green, London.
Schiefferdecker, P. (1909). "Muskeln und Muskelkerne." Barth, Leipzig. Quoted by Fernand (1949).
Schwalbe, G., and Mayeda, R. (1891). *Z. Biol.* **27**, 482.
Sichel, F. J. M. (1941). *Am. J. Physiol.* **133**, 446 P.
Sobotta, J. (1930). "Textbook and Atlas of Human Histology and Microscopic Anatomy," pp. 17, 97. Stechert, New York
Speidel, C. C. (1939). *Am. J. Anat.* **65**, 471.
Tawara, S (1906). "Das Reizleitungssystem des Saugethierherzens." Fischer, Jena.
Tsuruyama, K. (1937). *Japan. J. Med. Sci. I.* **6**, 249.
Van Breeman, V. L. (1953). *Anat. Record* **117**, 49.
Van Harreveld, A. (1947). *Am. J. Physiol.* **151**, 96.
Vinnikow, J. A. (1938). *Compt. rend. acad. sci. U.R.S.S.* **18**, 119.
Walls, E. W. (1945). *J. Anat.* **79**, 45.
Walls, E. W. (1948). *Brit Heart J.* **10**, 188.
Walls, E W. (1953). *J. Anat.* **87**, 437.
Wang, H. (1956). *Exptl. Cell. Research* **11**, 452.
Watzka, M. (1939). *Z. mikroskop anat. Forsch.* **45**, 668.
Wilkinson, H. J. (1929). *Med. J. Australia* **2**, 768.
Witte, L. (1919). *Am. J. Anat.* **25**. 333.

Clark, W. E. Le G. (1952). "The Tissues of the Body," pp. 144, 149. Oxford Univ. Press, London and New York.
Clark, W. E. Le G., and Blomfield, L. B. (1945). *J. Anat.* **79**, 15.
Cohn, A. E. (1909). *Verhandl. deut. pathol. Ges.* **13**, 182.
Cohn, A. E. (1932). *In* "Special Cytology" (E.V. Cowdry, ed.), p. 1159. Hoeber, New York.
Cohnheim, J. F. (1865). *Arch. pathol. Anat. u. Physiol. Virchow's* **34**, 606.
Davies, F. (1930). *J. Anat.* **64**, 129, 319.
Davies, F., and Francis, E. T. B. (1946). *Biol. Revs. Cambridge Phil. Soc.* **21**, 173
Davies, F., Francis, E. T. B., Wood, D. R., and Johnson, E. A. (1956). *Trans. Roy. Soc. Edinburgh* **63**, 71.
Denny-Brown, D. E. (1929). *Phil. Trans. Roy. Soc. London Ser.* **B 104**, 371.
Duckworth, J. W. A. (1952). The Development of the Sinu-atrial and Atrioventricular Nodes of the Human Heart. M. D. Thesis, University of Edinburgh. Quoted by Ham (1957).
Edwards, D. A. W. (1946). *J. Anat.* **80**, 147.
Eliot, T. S., Wigginton, R. C., and Corbin, K. B. (1943). *Anat. Record* **85**, 307.
Felix, W. (1887). "Festschrift für Albert von Kolliker," p. 282. Englemann, Leipzig. Quoted by Honcke (1947).
Fenn, W. O. (1945). *In* "Physical Chemistry of Cells and Tissues" (R. Hoeber ed.) p. 445. Churchill, London.
Fernand, V. S. V. (1949). The Sizes of Muscle Fibres and of their Nerve Fibres. Ph. D. Thesis, University of London.
Fischer, E. (1944). *Physiol. Revs.* **24**, 467.
Garven, H. S. D. (1925). *Brain* **48**, 380.
Graf, W. (1947). *Acta Psychiat. Neurol.* **22**, 21.
Haggqvist, G (1956). *In* "Handbuch der Mikroskopischen Anatomie der Menschen" (W. Bargmann, ed.), vol. 2, part 4, chap. 1, p. 4. Springer, Berlin.
Haines, R. W. (1934). *J. Anat.* **69**, 20.
Halban, J (1893). *Anat. Hefte* **3**, 267.
Hall, C. E., Jakus, M. A., and Schmitt, F. O. (1946). *Biol. Bull.* **90**, 32.
Ham, A. W. (1957). "Histology," 3rd ed., p. 390. Lippincott, Philadelphia, Pennsylvania.
Hammond, J., and Appleton, A. B. (1932). "Growth and Development of Mutton Qualities in the Sheep " Oliver and Boyd, Edinburgh
Harman, J. W. (1955). *Am. J. Phys. Med.* **34**, 68.
Harrison, R. G. (1947). *J. Anat.* **81**, 365.
Herrick, C. J. (1902). *J Comp Neurol.* **12**, 329.
Hogue, M J. (1937). *Anat. Record* **67**, 521
Honcke, P. (1947) *Acta Physiol. Scand.* **15**, Suppl. 48, 12.
Huber, G C (1916). *Anat. Record* **11**, 149.
Huxley, A. F (1957) *Progress in Biophysics*, **7**, 255.
Huxley, H. E (1957) *J. Biophys. Biochem. Cytol.* **3**, 631.
Jordan, H. E (1933) *Physiol Revs* **13**, 301
Jordan, H E , and Banks, J. B (1917). *Am. J Anat.* **22**, 285.
Jordan, H. E , and Steele, K B. (1912) *Am. J. Anat.* **13**, 151.
Karsner, H T , Saphir, O , and Todd, T W (1925). *Am. J Pathol.* **1**, 351.
Keith, A., and Flack, M. (1907). *J Anat* **41**, 172
Knoll, P. (1891). *Denkschr Akad. Wiss Wien* **58**, 633.
Kohashi, Y. (1937) *Okajimas Folio Anat. Japon* **15**, 175.
Kugler, J. H., and Parkin, J. B (1956) *Anat. Record* **126**, 335.
Kuhne, W. (1863). *Arch. pathol. Anat u Physiol Virchow's* **26**, 222.

CHAPTER III

Development of Striated Muscle

J. D. Boyd

I. SOURCE OF THE MESODERM CONCERNED IN THE DEVELOPMENT OF STRIATED MUSCLES

With the exception of the sphincter pupillae in birds and reptiles, the striated musculature of vertebrates is of mesodermal origin. The mesoderm concerned is part of that which, during gastrulation, is invaginated[1] through the primitive streak, and, in a very general manner, its source in earlier embryonic stages can be identified in the ectoblast. Neural crest mesoderm (ecto-mesenchyme) does not, apparently, contribute to the muscular system. On leaving the ectoblast, the invaginated cells, or chordamesoderm, become arranged into seven longitudinally arranged bands. Of these bands, that unpaired one which comes to lie in an axial position in the middle line of the embryo is the *notochord*. On each side of the notochord there is a band of *paraxial mesoderm*—most of which becomes segmented to form the somites. Along the outer borders of these somites are found the more attenuated strands of *intermediate mesoderm*. Finally, and lying most laterally on each side, is the *lateral plate mesoderm*. The appearance of the coelom splits each lateral plate tangentially into an outer part, in contact with the ectoderm and known as the *somatopleuric mesoderm*,

[1] Although the tail mesoderm is not invaginated in the manner of that destined for more cranial regions of the body, there would seem, in fact, to be no specific and essential differences in the method of development of the middle germ layer along the embryonic axis. Thus the presumptive material for the urodele tail somites has been identified in the hindmost part of the ectoblast of the "neural plate" (Bijtel, 1958), and there seems to be no justification for regarding the tail as arising by a process essentially different from that obtaining elsewhere in the embryonic axis.

superficial muscles of the face, the auricle, and the scalp have evolved (Huber, 1931) and from which, presumably, they develop.

The pharyngeal muscles, in the form of the constrictors, extend to the commencement of the esophagus. In the walls of the latter, in most vertebrates, there is a variable amount of striated muscle which sometimes reaches as far caudally as the cardiac orifice. Indeed, in some fishes, this striated musculature is found to extend to the wall of the stomach itself. Such postpharyngeal striated gut musculature is of uncertain embryological origin, but presumably it is developed *in situ* in the splanchnopleuric mesoderm; it can, therefore, be considered to be of general visceral origin. Its nerve supply has not been established in detail, but seems to resemble that to striated musculature elsewhere.

The striated musculature of the paired extremities of vertebrates is certainly of mesodermal origin. In the paired fins of nontetrapods the contained musculature takes its origin from ventrolateral extensions of the myotomes into the mesenchyme of the developing fins. In tetrapods, however, with the possible exception of reptiles (Dalcq and Pasteels, 1954, p. 197), the evidence for such myotomic extensions is not convincing. Indeed many descriptive embryologists (Häggqvist, 1956, for references) and most investigators who have used experimental techniques (e.g. Byrnes, 1898; Lewis, 1910; Detwiler, 1934, 1955; Hamburger, 1938, 1939; Saunders, 1948b; Tschumi, 1957) consider that the limb muscles differentiate *in situ* from the limb bud mesenchyme. This mesenchyme is of somatopleuric origin. The prospective mesenchymal cells of the limb buds make their first appearances as localized aggregations of somatopleuric cells near the coelomic epithelium. From this situation these cells migrate to the covering epidermis, which is somewhat thickened opposite to them, and establish contact with it (Filatow, 1933; Taylor, 1943). The protuberance which results from the proliferation of these somatopleuric mesodermal cells and their epidermal covering constitutes the limb bud. The evidence suggests strongly that it is only through multiplication of the constituent cells of the bud that its subsequent growth can ensue. There is no evidence for further supply of mesodermal material from any other source, including the myotomes. With the exceptions of the nerve fibers, the Schwann cells, the melanoblasts and, possibly, the endothelium of the vascular and lymphatic systems, all the subepidermal cellular elements of the limbs, including those of the muscles, are now generally considered to take their origin *in situ* from

and an inner part, which clothes the endoderm and is called the *splanchnopleuric mesoderm*. Muscular tissue, as will be described, arises only from paraxial and lateral plate mesoderm.

II. REGIONAL DIFFERENCES IN ORIGIN OF THE STRIATED MUSCULATURE

The striated musculature of the trunk, which is the first to appear in ontogeny, is derived, at least in great part, from the myotomic portions of the paired and segmentally arranged somites. In the head and neck region, striated muscle of somite origin is also present. Thus the extrinsic muscles of the eyes arise from three pre-otic somites or from a localized and condensed mass of mesoderm which corresponds to them. The tongue musculature, innervated by the hypoglossal nerve, also takes its origin, at least phylogenetically, from the so-called occipital somites. (See R. McM. Hunter, 1935; R. P. Hunter, 1935.) The cervical somites, of course, provide the material for the pre- and postvertebral musculature in the neck. Definite evidence for a myotomic origin of the infrahyoid muscles is not forthcoming, but such a derivation for these muscles is generally assumed. In addition to this somitic, or myotomic, musculature, there is, in the vertebrate head and neck region, an extensive system of voluntary striated musculature derived from the visceral, or branchial, arch mesoderm. This segmentally arranged "special visceral" musculature constitutes that of the jaws, of the pharynx and, in branchiate vertebrates, of the gills. In the air-breathing vertebrates the first visceral arch remains the source of the masticatory muscles; the myogenic tissue of the arches caudal to the first develops principally into the musculature of the pharynx and the larynx.[2] In the amniotes the second arch mesoderm also provides an extensive sheet of superficial musculature known as the *sphincter colli*. In the mammals, an extension of the last named muscle in a cranial direction constitutes the matrix out of which the

[2] There are in most vertebrates 6 branchial arches, and the muscles derived from each of them are supplied by the appropriate branchial arch nerve. The sternomastoid and trapezius muscles, supplied by the accessory nerve, are probably also to be regarded as, at least in part, of branchial origin, possibly phylogenetically from branchial mesoderm lying caudal to the sixth arch. W. H. Lewis, indeed, considered that there is no somitic contribution to the sternomastoid and trapezius muscles, [illegible]

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the mesenchyme of their embryonic buds. Thus it has been shown by Hamburger (1938, 1939) and by Rudnick (1945) that, in stages before it has become a distinct protuberance, the forelimb primordium of the chick can undergo self-differentiation into an essentially complete and normal wing when grafted to the coelom of a host chick. Indeed, subsequent work on the chick wing bud by Saunders (1948a) and on the hind-limb bud of *Xenopus laevis* by Tschumi (1957) has shown quite conclusively that, in the animals concerned, the most actively growing part of the limb bud is its distal end. Rapid proliferation of this apical mesenchyme has as its result the continuous laying down of the limb segments in a proximo-distal manner. If the apical growth is interrupted, the distal part of the limb lacks its mesodermal constituents, the extent of the lack depending on the stage of interference and the intensity of the interrupting trauma. The results of these experiments leave little doubt of the reality of *in situ* differentiation of those muscles which are properly intrinsic to the limbs. The descriptive work of many investigators suggests strongly that many of the muscles of the pectoral and pelvic girdles are of myotomic origin. There is, to my knowledge, no clear-cut experimental evidence on this point, and meanwhile it would seem that we should consider the region of transition, at the proximal end of a limb, as one in which there is an indefinite transition between musculature of myotomic and musculature of *in situ* limb bud origin. Probably, in later stages of development, muscle from limb can extend into trunk and *vice versa*. Such exceptions, however, should not be taken to transgress the general conclusion that the limb muscles of birds and amphibia certainly, and probably of all tetrapods, are not of myotomic origin. The careful and extensive histological investigations of Streeter (1942, 1949) and further work by Theiler (1957) on human embryos add weight to this conclusion.

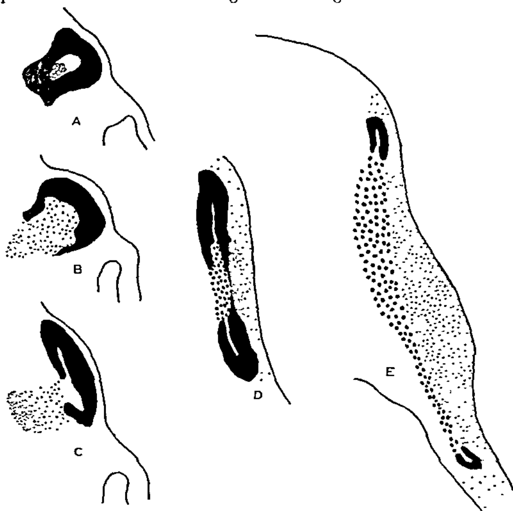
A case for another exception to the myotomic origin of trunk muscle has been made by Straus and Rawles (1953). As early as 1868 His denied a contribution by the somites to the abdominal musculature which, in his opinion, probably arose from the somatopleuric portion of the lateral plate From a study, on the chick embryo, of the results of carbon marking experiments and of the fate of intracoelomic grafts of lateral plate material, Straus and Rawles conclude that the ventral parts of all three lateral abdominal muscles, the whole of the rectus abdominis muscle, and the ventral parts of the intercostal muscles develop from mesodermal cells of the lateral plate. Their generalized

conclusion for the chick is that "the somites alone form the skeleton and musculature of approximately the dorsal third of the body wall; the lateral plate alone forms these structures in approximately the ventral half. The intervening area, representing approximately one-sixth of the body wall, appears to be formed by both somites and lateral plate." In their paper, Straus and Rawles indicate that, although the situation is not entirely clear, it appears likely that in urodeles also at least some of the ventro-lateral trunk musculature is derived from the lateral plate. "Unfortunately," they add, "comparable data are lacking for other classes of vertebrates." However, in 1955, Detwiler produced cumulative evidence from a number of differently executed experiments on *Amblystoma* larvae suggesting strongly that in these urodeles the ventrolateral trunk musculature is, in fact, of somite origin. More recently still, Theiler (1957) has made a careful study of serial sections of 26 human embryos ranging from 2.5 mm. to 13 mm. C. R. length, and has compared his findings with sections of chick, guinea pig, pig, and mice embryos. Theiler finds that ventral extensions of the somites, which he calls somitic buds (*Somitenknospen*), can be traced, in human embryos, of 5–9 mm. C.R. length, into the lateral plate mesoderm between the levels of the eighth cervical and the second lumbar segments (Text Fig. I, E). These buds represent the undifferentiated ventral edges of the myotomes. They develop in accordance with the law of cranio-caudal differentiation and they could well be the source of the intercostal and ventral abdominal musculature. Theiler concludes that his findings "are not contradictory to the theory of a somitic origin of the intercostal and abdominal musculature." It would seem, therefore, that the case for a lateral plate origin of the ventrolateral trunk musculature cannot be regarded as proven. It is, of course, possible that the developmental situation in the chick embryo differs radically from that in urodeles and mammals.

III. DEVELOPMENTAL HISTORY OF THE MYOTOME

In gnathostomatous vertebrates there are marked differences, both from species to species and regionally in a single species, in the details of the developmental history of the paraxial somites. The general pattern, however, is that the medial wall and a considerable part of the cranial and caudal walls of each more or less cubical somitic block (Text Fig. I, A) resolve themselves into a loose mesenchyme. As

the so-called sclerotome (Text Fig. 1, B), these mesenchymal cells migrate ventromedially round the notochord, where they meet and fuse with the equivalent cells from the opposite side. From these sclerotomic cells the vertebrae and the dura mater will develop. The portion of the somite remaining after the migration of the sclerotomic



Text Fig 1 Successive stages in development of somite and myotome. A; Left fifth somite in a 10 somite human embryo. The somitic wall is solid black. There are some cells loosely arranged in the cavity of the somite. B; Left sixteenth somite in a 28 somite human embryo. The medial wall of the somite has resolved itself into a loose mesenchyme which, as the sclerotome, is streaming medially. C; Left tenth somite in a 28 somite human embryo. The original ventral and dorsal walls of the somite are bent towards the intact lateral wall to constitute the myotome. D; Left midthoracic myotome in a 5 mm. human embryo. The lateral myotomic wall is now interrupted and the dermis has appeared. E; Left midthoracic myotome in an 8 mm. human embryo The remaining epithelial-like dorsal and ventral margins of the myotome are now widely separated, the ventral margin having extended into the somatopleure.

material is called the myotome (*Hautmuskelplatte* or dermomyotome). This myotome consists of the lateral wall and of parts of the dorsal and ventral walls of the original somite. The cells of these persisting walls have an epithelial-like appearance, those of the lateral wall in particular taking on a high cylindrical character. As a result, the general appearance presented, on section, by the myotome at this stage is of an epithelial shell with a medially directed concavity. The surface of the shell lies immediately beneath the ectoderm, no mesenchymatous tissue being interposed. For a period after the separation of the main mass of the sclerotome the medial concavity, or hilum, of the myotome often still shows some mesenchymal cells migrating from it (Text Fig. 1, C). Meanwhile, the persisting part of the dorsal wall comes to apply itself, with varying degrees of intimacy of contact, to the inner surface of the intact outer somitic wall which is now called the dermatome (*Cutislamella* or *feuillet aponévrotique*). The shell thus becomes in part double layered. The persisting part of the ventral wall, which is distinctly shorter than the dorsal one, will eventually come similarly to oppose itself to the outer wall. For some time, however, the ventral wall appears as a medially directed projection of the myotome. Possibly the differentiation of the dorsal root ganglia and of the associated neural crest material exerts more pressure dorsally. This would explain the temporal difference in behavior between the dorsal and ventral somitic walls. By the time these two walls have come into contact with the dermotome, the central part of the latter shows a breakdown of its epithelial character (Text Fig. 1, D) and mesenchymatous cells migrate from its outer surface and come to lie between it and the overlying ectoderm. Previous to the appearance of these cells, there has been no cellular tissue interposed between the ectoderm and the lateral somitic wall. The mesenchyme derived from the dermatome is considered to constitute that of the dermis, at least in the immediate neighborhood of its origin. The gap in the dermatome, resulting from its central breakdown, rapidly extends to the cranial and caudal limits of the lateral wall, so that the persisting epithelial parts of this wall come to be separated into a dorsal and a ventral moiety. It is generally believed that the dermatome gives rise only to mesenchyme. Bardeen (1900) considered that, in the pig, it develops exclusively into muscle, a view that is certainly mistaken. Graham Kerr (1919), however, states categorically that, in *Lepidosiren*, it gives origin to some striped muscle.

The main mass of muscle of somite origin arises from the inner layer of the myotome or from cells proliferated from this layer. In anamniotes, the cells of the inner layer tend to enlarge considerably and to orientate themselves in a cranio-caudal direction. Eventually, many such elongated and hypertrophied cells extend throughout the whole length of the myotome. They become multinucleated and eventually myofibrils appear in their cytoplasm. In the amniotes the differentiation of the inner myotomic wall is not so easy to interpret. In the first place, in these vertebrates this wall is frequently never quite complete since the dorsal and ventral walls of the original somite frequently do not meet and fuse. Indeed, the spatial separation of the dorsal and ventral portions of the dermatome is often accompanied by an apparent stretching of the two components of the medial wall (compare Text Fig. 1, A and D). There also appears to be more mitotic activity in the myotomes of birds and mammals than in fishes or amphibia and the cells of the inner layer consequently remain smaller for a longer time. Nevertheless, in the amniotes there is a cranio-caudal elongation of some of the cells of this layer, and eventually myofibrils will appear in them. Moreover, in the amniotes, the persisting dorsal and ventral portions of the original myotome in retreating from each other have laid down more cells, some of which will become myoblasts while others will probably form mesenchyme and fibroblasts which may be concerned in the development of tendon (Butcher, 1933). The appearances resulting from these histogenetic processes in the myotomes of the higher vertebrates are very confusing and require further elucidation. Nevertheless, it is apparent that, in these vertebrate types also, a great mass of striated muscle is produced by the inner wall of the myotome, either directly or through the production of cells which are small enough to migrate and which then differentiate into muscle in positions remote from the myotome itself. Such complications in myotomic differentiation in the amniotes are, perhaps, to be expected in view of the greater diversity in the locomotor apparatus and the muscular system of these vertebrates.

As has been indicated earlier, the prospective somite material in a number of vertebrates has been identified in the ectoderm before gastrulation. Yamada (1937, 1939), however, showed that, in amphibia, if such prospective material is grown within epidermal jackets or is transplanted into the midventral belly region, it does not develop into striated muscle but into pronephric tubules. These findings

suggested that, for its normal differentiation, somite material requires the intervention of some extrinsic factor or factors. Yamada (1939), Muratori (1939), Mutchmore (1951), and Smithberg (1954) have all demonstrated that the notochord probably contains such factors, for when it is explanted in conjunction with presumptive somite material, the latter can differentiate into striated muscle. The notochord is not unique in this regard since other tissues can substitute for it. Mutchmore considers that the experimental results suggest that "muscle differentiation is dependent upon the maintenance of a certain proportion of the normal relationships of the developing somite to surrounding tissues." Strudel (1955) has also produced evidence that the presence of the spinal cord and notochord is necessary for differentiation of the vertebrae and of the vertebral muscles in the chick embryo. More recently still, however, Holtzer *et al.* (1956) have reported experiments suggesting that spinal cord, in amphibia and in birds, stimulates the growth of somitic muscle, whereas the notochord is inert. Avery *et al.* (1956) also consider that chick notochord does not enhance the growth of somitic muscle.

Whatever local factors are concerned in the differentiation of embryonic muscle, the presence of a nerve supply to it is not one of them. The absence of an effect on this differentiation by the peripheral nerves was first demonstrated by Ross Harrison (1904) and his work has been amplified by, among others, Lewis and Lewis (1917), Hamburger (1929), Szepsenwol (1947), and Avery *et al.* (1956).

IV. THE MYOBLAST

The term myoblast has been used in different ways. For Godlewski (1901), who introduced the term into general use, it meant those embryonic elements which, differing little from the remaining embryonic cells, eventually become muscle fibers. As Katznelson (1936) pointed out, such a definition is unclear and is, in practice, not very helpful. Tello's (1917, 1922) classification of the stages between the undifferentiated embryonic cell and the fully developed muscle fiber is much more fruitful. Tello identifies the following stages in the embryology of the muscle fiber: (1) that of the *myoblast*, in which the primordial muscle cell cannot be distinguished from the associated fibroblasts; (2) that of the *myocyte*, in which the cell, which may be uni- or multinucleated, has become elongated but shows no transverse striation and no specific cytoplasmic structure; (3) that of the *myotube*, in which the very

elongated cell shows some striation in its periphery and an appearance of an axial core of pale cytoplasm, with the nuclei still situated centrally; and (4) that of the *muscular fiber*, with fully established transverse striation and nuclei situated in the periphery. Katznelson uses the term myoblast for both of the first two stages in Tello's classification. Indeed, in descriptive histology, until the criteria laid down by Tello for myocytes have become apparent, it is only by position, supported by the eye of faith, that a cell can be identified as a myoblast in Tello's stage 1. Further, the use, for cells in the state of development corresponding to Tello's stage 2, of the term myocyte suggests a completion of development which they patently have not achieved. In my opinion, the better terms for Tello's stages 1 and 2 would be, respectively, premyoblast and myoblast. It is in this way that the terms will be used in the subsequent discussion.

V. HISTOGENESIS OF STRIATED MUSCLE

There has been much conflict of opinion on the manner in which the myoblasts become converted into the elongated, multinucleated, and highly specialized muscle fibers. As early as 1839, Schwann expressed the opinion that, in the pig, the definitive functional fibers of muscle arose by the alignment of the formative elements into parallel rows in which the cells were arranged in an end-to-end fashion. He considered that linear coalescence of these cells produced the elongated multinucleated condition. Schwann's view was soon contested by Prévost and Lebert (1844) and by Remak (1845), who believed that each muscle fiber was derived, by growth and nuclear division, from a single embryonic cell. In the subsequent hundred years, many investigators (Tello, 1922; Haggqvist, 1931, 1956, for summaries of the literature) have reported findings which support one or other of these viewpoints. This literature is confusing and an attempt at an impartial assessment of it leads to two conclusions. In the first place, it is apparent that the material used in many of the contributions was not adequate in amount or in histological state, for a solution of the problem. Secondly, it is possible that all the striated muscle fibers of vertebrates do not undergo identical histogenetic processes. Thus myoblasts derived from the epithelial-like cells of the somites may behave differently from those which arise *in situ* from mesenchyme. Further, Glücksmann (1934) has described striking differences in the development of muscle in different vertebrate groups. Indeed, as will

be seen later, there may even be differences in the mode of origin of muscle fibers at different stages in the development of a single muscle. The following account is based principally on observations on human and sheep embryos; examination of more limited material from other vertebrate types, however, does not lead to the opinion that the histogenesis of muscle in them differs in any essential manner from that which is found in mammals.

In the earliest musculature arising directly from the dermomyotome, the premyoblasts are represented by the elongated epithelial cells of its inner wall. In later stages, in this position and in all those regions where the musculature arises *in situ* in mesenchyme, the premyoblasts are slightly elongated. Apart from this elongation of the premyoblasts, however, it is only by their position in relation to the subsequent developmental history of the region concerned that they can be identified as myogenic in character. Like the closely related cells of fibroblastic nature, these premyoblasts show mitotic activity. As is usual in mitosis, the dividing cells become round and, hence, during division, there is also no method of distinguishing premyoblasts from fibroblasts. With continuing development, however, cells that can be identified as myoblasts appear in the regions of myogenesis. These cells are characterized at first by their larger size, by their greater elongation, and by an absence of mitotic activity. Their cytoplasm increases considerably in amount; it is granular and often vesicular in character. Owing to the initial tight packing of the myoblasts and to the diffuse nature of their cytoplasmic boundaries, their increase in size and the vacuolation of their cytoplasm often results in an appearance as if there had been cell fusion, and not by cell ends only, to constitute a widespread syncytium. There are many descriptions in the literature indicating that there is, indeed, a true syncytial formation by the myoblasts. Careful study of the regions of alleged cytoplasmic fusion leaves one undecided on this important point. In well fixed material, cell boundaries can, in fact, usually be identified (Plate III, Fig. 13). However, even in embryos in which fixation in general is excellent, the myoblasts frequently show such marked vacuolation and indeterminancy of bounding cytoplasm (see Plate I, Figs. 1 and 3) that it would be rash to state dogmatically that there has been no fusion. In view of the subsequent history of the myoblasts, syncytial formation is, perhaps, not to be expected. The appearances of fusion of the myoblasts are possibly due to a peculiar lability of their cytoplasm to fixatives at

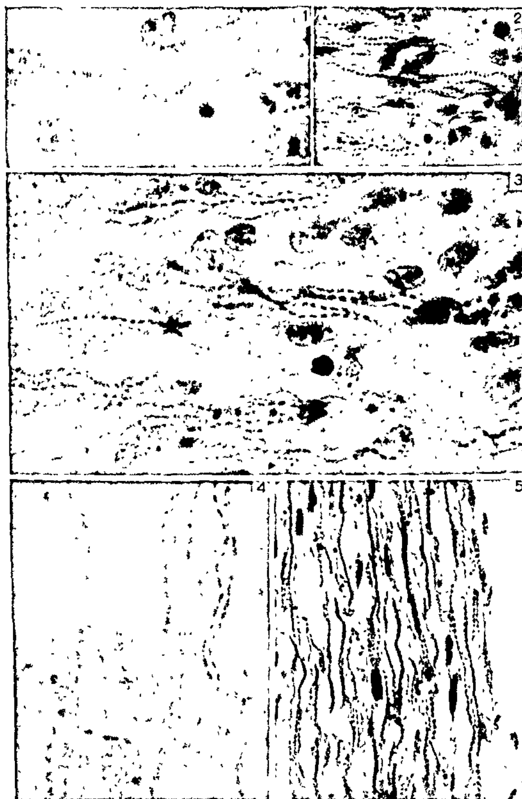


PLATE I

this stage. Glücksmann (1934) and Ballas and Obert (1952) describe the syncytial appearance as being found only in the higher vertebrates.

The granularity of the cytoplasm of the myoblasts at this stage is in part due to mitochondria, as was first indicated by Meves (1907) and Duesberg (1909) and as has more recently been demonstrated in developing chick somite muscle by Weed (1936). It is also, in part, attributable to the presence of slightly eosinophilic bodies which may correspond to the cytoplasmic granules described by Moscona (1955) in embryonic chick myogenic cells growing *in vitro*. Moscona describes these granules as ranging from 0.8–2.2 μ in diameter. They do not stain supravitally with Janus green under conditions which demonstrate mitochondria and they are too large for microsomes. They appear to contain a polysaccharide in association with a protein and, also, ribonucleoprotein.

As development proceeds, the myoblasts become much more elongated and, at the same time, they increase in girth. Any appearance of syncytial formation which may have been present disappears. The resulting cells become multinucleated, apparently by amitotic division, for no mitotic figures are observed. The nuclei are situated centrally and frequently two, three, or more of them are situated close together in a linear fashion. The absence of peripherally situated nuclei is, perhaps, a point against the origin of the myoblasts of this stage from a diffuse syncytium. With the assumption of the multinucleate condition, the cytoplasm of the central portion of each myoblast becomes much clearer and the general granularity of the remaining

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FIG. 1. Section through developing trunk muscle in 28 day (6 mm. C.R. length) sheep enbryo. A number of homogeneous and several striated myofibrils can be seen. Note the absence of obvious cytoplasmic boundaries. Iron hematoxylin. Magnification: × 1060.

FIG. 2. Section through developing trunk muscle in a 17 mm. C.R. length human embryo (H. 640). Striated myofibrils can be seen in the myoblastic cytoplasm. Iron hematoxylin. Magnification: × 560

FIG. 3. Higher power photomicrograph of trunk muscle in 17 mm. C.R. length human embryo, showing striated myofibrils in vacuolated cytoplasm. A *pyknotic* nucleus is present, such presumably degenerating nuclei are not uncommon at this stage. Iron hematoxylin. Magnification: × 1050.

FIG. 4. Myofibrils in a 17 mm. C.R. length human embryo, showing longitudinal splitting in several regions. Iron hematoxylin. Magnification: × 1540.

FIG. 5. Myotubes in trunk muscle of a 22 mm. human embryo (H. 594). Iron hematoxylin. Magnification: × 532

cytoplasm is decreased. In the central clearer cytoplasm, however, there is now present particulate glycogen and also some lipid (Couteaux, 1941). It is at this stage (5–8 mm. in man and sheep, third day in chick) that the myofibrils can first be identified in the myoblasts of those muscles which are more advanced in their development.

There have been widely differing opinions on the origin of the myofibrils. The earlier investigators considered that they differentiated out of homogeneous myoblastic protoplasm. Godlewski (1901, 1902) described them as arising through linear aggregation and fusion of initially randomly arranged protoplasmic granules. Moscona (1955) has also drawn attention to the origin of the myofibrils at that time when the cytoplasmic granules described by him disappear. Meves (1907) and Duesberg (1909) have associated mitochondria with the initiation of myofibrillar development but Häggqvist (1920) did not agree with them. Weed (1936), in her study on myogenesis in the chick, has associated both mitochondria and granules in the fibrillogenesis. She writes: "The myofibrils were found to arise in close association with filamentous mitochondria, apparently at the expense of the numerous small cytoplasmic granules which fill the early myoblasts." Most observers are agreed that, when they first appear, the individual myofibrils are homogeneous and stain very palely with iron hematoxylin and that only one or two of them can be identified in each cell. Later they increase in number, stain much more deeply, and eventually show the appearance of striation. Godlewski (1902) considered that the anisotropic band of this striation corresponded in nature to the original granules, from which he believed the myofibrils to be derived. When they are in the homogeneous state, the myofibrils are very attenuated. With the appearance of striation, however, their diameter averages, as measurement of a large number of them shows, about 1 μ. It can be recorded, too, that the anisotropic and isotropic segments possess approximately their definitive lengths as soon as they become apparent. Thus, measurements of 100 A segments in a 17 mm. human embryo gave an average length for these segments of 1.4 μ with no measurements falling outside the range of 1.3–1.5 μ. The equivalent average for the I segments is 0.8 μ, but the range is rather wider. Nevertheless, the dimensions are strikingly of the same order as in adult muscle. The differentiation of the myofibrils commences in their intermediate portions and thence spreads rapidly along their length towards their ends. As the myoblast grows, it would seem that,

initially at any rate, new terminal parts of each myofibril consist of homogeneous material into which the striation rapidly extends. The regularity in the periodicity of the intermediate portions of the myofibrils would seem to argue strongly against any interstitial growth in them during their elongation.

When the myofibrils first appear and are in the homogeneous state, each myoblast contains only a very small number of them. Of these, one appears to become striated in advance of the others. Often before these others develop their striations, the more advanced myofibril begins, in its intermediate section, to divide longitudinally. Indeed, this myofibril may commence to divide in its central part while its two ends are still in the homogeneous condition. The duplication of the myofibrils by longitudinal splitting was first described by Heidenhain and by Maurer in 1891. Many subsequent observers have verified their descriptions. In developing trout muscle, Heidenhain (1913) described all the myofibrils in a myoblast as arising from one single longitudinal cytoplasmic fibril by a rather complicated process of splitting. Unfortunately, there appear to be no recorded observations on the cytological conditions in trout muscle antecedent to the formation of this single primary fibril. In mammalian myoblasts, it seems unlikely that the myofibrils are all derived from a single forerunner, unless the homogeneous fibrils have had such an origin before they become thick enough to be resolved by light microscopy.

During the process of division of a striated myofibril, the darkly staining anisotropic bands, as they appear in sections stained with Heidenhain's iron alum hematoxylin, become paler. The longitudinal separation would appear to be the result of an actual split in the middle of the fibril; the two resulting fibrils each possess about half the diameter of the original fibril. Then, and apparently quite rapidly, the components of each daughter fibril swell to their original thickness. Production of new myofibrils in this fashion proceeds for a long period—in man, certainly until the middle of intrauterine life. It becomes progressively more difficult to observe the details of the process of splitting, however, as the myoblast comes to contain more and more myofibrils. During the process of multiplication of the myofibrils, equivalent isotropic and anisotropic segments keep in register. This phenomenon is not surprising for those myofibrils which have had their origin from a common forerunner. It is not apparent, however, what mechanism brings into register equivalent segments of

myofibrils of disparate origin. Possibly the arrangement is due to a common origin of the homogeneous fibrils from a single initial fibril in the earliest stage of myoblastic differentiation. Weed (1936) has suggested that the Z-membranes may be present from the time the fibrils first appear and their early presence might be the explanation for the alignment of equivalent bands. I have been unable to identify with any feeling of certainty Z- membranes at such early stages. As Heidenhain (1913) has shown, there are occasional lapses in the correctness of register.

While the myofibrils are differentiating, there are other striking changes in the myoblasts which result in their gradually passing into the *myotube* stage. The nuclei are still situated centrally; they tend to be rectangular in shape except in those regions where several of them are closely apposed to each other and where, presumably, they have been undergoing a mitotic division. The cytoplasm between the nuclei, in the central region of the developing muscle cell, stains very palely in iron hematoxylin material. With continuing development, this paleness of the axial cytoplasmic core becomes more marked and the general appearance presented by a section of any length through the cell is of a tubular structure. With suitable fixation and staining, it is possible to identify both P.A.S. positive material and lipid granules in the axial cytoplasm of these myotubes. The P.A.S. material is largely diastase sensitive, but even after long subjection to this enzyme, some positive reaction persists. There can be no doubt that much of the central pallor in myoblasts subjected to routine histological techniques is due to the removal from the axial cytoplasm of water- and alcohol-soluble substances.

Fig. 6 Higher power view of longitudinal section through myotubes with myofibrils in 22 mm C.R length human embryo (H. 594). Iron hematoxylin. Magnification × 1050

Fig 7 Longitudinal section of myofibrils in trunk muscle of a 22 mm. C.R. length human embryo Several of the myofibrils are splitting longitudinally. Just above the middle of the field, a split myofibril can be followed (to the right) to a single striated myofibril which, in turn, can be traced into a fine homogeneous fibril. Iron hematoxylin Magnification × 1050.

Fig 8 Transverse section of myotubes in developing sternohyoid muscle of a 43 mm C.R length human embryo (H 591). Four myotubes show the appearance of marked siderophilia in the thickened peripheral cytoplasm. Iron hematoxylin. Magnification × 397

Fig. 9. Higher power view of part of section shown in Fig. 8. Note peripheral situation of myofibrils in the myotubes and the clearness of the central cavity except where nuclei are present. Iron hematoxylin. Magnification: × 900.



PLATE II

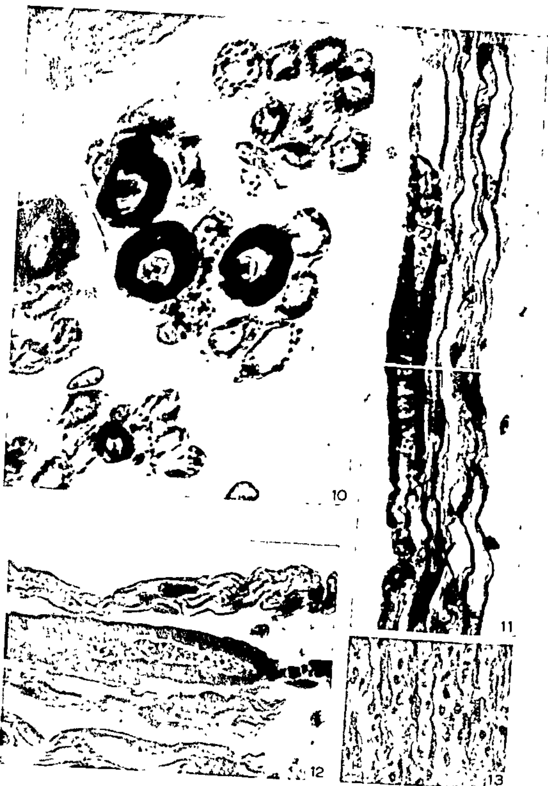


PLATE III

In the myotubes the myofibrils are arranged concentrically (Plate II, Fig. 9; Plate III, Fig. 10) around the pale axial core in the denser peripheral cytoplasm. As development proceeds, many of the myotubes show a very remarkable alteration in part of their periphery. This alteration is usually found at about the middle of a myotube's length, and it consists in a thickening of the cytoplasm accompanied by a striking increase in affinity for iron hematoxylin. Indeed, it is only occasionally that the myofibrils can be identified in the general darkness of this cytoplasmic segment. At this level the nuclei of the myotube tend to be arranged in closely packed longitudinal formation (Plate III, Figs. 11–12). There are no signs of any cellular reaction, such as might be expected if the change is a pathological one, outside of the myotubes in the regions adjacent to the cytoplasmic alteration. Certainly there is no sign of phagocytosis. Moreover, if one passes along the length of a myotube showing this alteration, after a region of transition showing irregular signs of the change, one comes to perfectly normal myotube structure (Plate III, Fig. 11). This alteration has frequently been described as evidence for degeneration in developing muscle (Häggqvist, 1931; Glucksmann, 1934, 1951). It has also been suggested that it may represent regions of contracture (Couteaux, 1941). If it were not for the fact that the myofibrils do not seem to show interstitial growth, it would be tempting to suggest that perhaps these altered regions in the length of a myotube represent regions of growth in length of the developing cell. The alterations were found in muscles from all parts of the body of both human and sheep embryos. They

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FIG. 10. Transverse section of myotubes in the developing sternomastoid muscle of a 43 mm. C.R. length human embryo (H. 591). Note the extreme density of staining of the peripheral cytoplasm in the larger myotubes. Myofibrils are well shown in most of the other myotubes. Iron hematoxylin Magnification · × 1050.

FIG 11. Longitudinal section through the pectoralis major muscle of a 43 mm. human embryo. The myotube on the left shows the altered segment with closely apposed nuclei and dense peripheral cytoplasm. The dense staining becomes patchy as the fiber is followed upwards and downwards and is eventually replaced by myotube of normal character. Adjacent myotubes are apparently unaffected by the proximity of the highly modified myotube. Iron hematoxylin. Magnification. × 520.

FIG. 12. Longitudinal section through part of modified segment of a myotube in the gluteus maximus muscle of a 43 mm. C.R. length human enbryo. Iron hematoxylin. Magnification: × 675

FIG. 13. Longitudinal section through developing somite muscle of an 8 mm. sheep embryo, showing myoblasts and interstitial cells. The myofibrils are mostly homogeneous and the myoblasts posses clear cut boundaries. Magnification: × 104.

seem to occur more frequently in the large muscles (e.g. the pectoralis major, gluteus maximus, and the sternomastoid) and they were only rarely found in the tongue. The changes in the human material examined are less frequent after the 100 mm. stage. If the changes are degenerative in nature and result in complete destruction of the associated myotube, the products of that degeneration must be removed with remarkable speed, for in a large and closely spaced collection of human embryos and fetuses, such products have not been identified. Whether or not all the myotubes show the phenomenon at some stage of their development, I cannot say.

Gradually, and at widely differing rates in different muscles and even in different parts of the same muscle, the myotubes are converted into muscle fibers. The process commences in the central part of a myotube with the disappearance of the pale axial cytoplasmic core. The nuclei become spaced out in a regular fashion and migrate to the margins of the fiber and come to lie immediately under the sarcoplasmic membrane. No signs of the formation of a sarcolemmal sheath by the fiber itself have been observed. With the disappearance of the tubular structure and the peripheral migration of the nuclei, the now very numerous myofibrils come to occupy the central part of the muscle fiber. Multiplication of the myofibrils appears to continue for a long time, as the fiber itself seems to increase in girth more or less progressively during gestation.

VI. INCREASE IN NUMBER OF MUSCLE FIBERS DURING DEVELOPMENT

It has long been recognized that the number of fibers in a muscle increases considerably in the later stages of embryonic and during fetal development. Thus MacCallum (1898) found that in the human sartorius muscle there is a twentyfold increase between the 74 mm. C.R. length stage and full term. Morpurgo (1898) made a similar observation on the muscles of developing rats. There have been two explanations for this marked increase. Firstly, a number of observers consider that the new fibers arise by the splitting of (or even by budding from) already differentiated striated fibers. Godlewski (1902), Ciardi-Dupré (1938), and Cuajunco (1940, 1942) have described such splitting and Tello (1922) figured a section through an altered central sector of a myotube, very similar to those shown in Plate II, Figs. 8–9, and Plate III, Fig. 10, which he considered was evidence for such division (*Myotubenvermehrung*). Couteaux (1941), however, has added

considerable support to those who consider that the augmentation in the number of muscle fibers is due to recruitment by the differentiation of interstitial, fibroblastic-like cells related to, and surrounding, the primary muscle fibers. In stages of development before the appearance of muscle fibers of this second generation, the "primary" myotubes are covered with numerous uninucleated elements which multiply on their surfaces by mitotic division. (Plate III, Fig. 13). In Couteaux's opinion, it is from some of these satellite cells that new fibers are added to the developing muscle until the full number is achieved. Personal observations strongly support this interpretation.

Whatever the method of development of the new muscular fibers which are added to a muscle during its development, the appearance presented on section of the muscle in the late embryonic and in the fetal period is of marked disparity in transverse diameter of the fibers. The appearances, indeed, correspond closely with Wohlfart's (1937) account of human muscle, in which he considered that two varieties of fiber could be distinguished. In each "primary" muscle bundle, Wohlfart described a thick "b-fiber" round which were grouped a number of thinner "a-fibers." This appearance was found first in the fourth fetal month and could still be seen, in the sartorius muscle, in the second year of postnatal life. Wohlfart concluded that there was a functional difference between the two types of fiber. It seems more likely, however, that they are the result of differences in time of assumption of structural maturity.

VII. DEVELOPMENTAL RELATIONSHIP OF MUSCLE AND TENDON

There has been much discussion of this relationship. Many investigators (most notably Schmidt, 1927) have described a direct continuity of the myofibrils through a transitional zone (*Uebergangsgebiet*) with fibrils in the substance of the associated tendon. A careful study, particularly of trichrome stained material, leads one to doubt if the relationship is more than that of contiguity. While it is possible to observe some branching of the terminal cytoplasm of a muscle fiber as it ends in a tendon or in loose connective tissue, in well-stained material no sign of segmented myofibrils transgressing the end of a fiber has been observed by the author. Indeed, a study of the histogenesis of muscle leads one to consider that in the embryo it is formed of two components. There is firstly the cells of the mesenchymatous background which become the fibrous tissue of the muscle and which

probably give origin to the endomysial (sometimes called sarcolemmal membrane; these satellite fibroblastic cells and their connective tissue derivatives are directly continuous with the tendon, where there is one. The other element is the contractile one, and is constituted by the primary myoblasts, reinforced in later stages by the recruits from the satellite cells. Couteaux (1941) apparently would go even further than this conclusion for he writes: "Il semble, en effet, que . . . le tissu musculaire strié et le tissu tendineux se comportent au cours du développement musculaire comme des tissus absolument distincts." Certainly the distinction is clear enough to justify the opinion that the contractile and the connective tissue elements in a muscle and its tendon constitute a composite organ rather than a single uniform tissue.

References

Avery, G., Chow, M., and Holtzer, H. (1956). *J. Exptl. Zool.* **132**, 409.
Ballas, W. F., and Obert, S. C. (1952). *Biologica (Santiago)* **14**, 15.
Bardeen, C. R. (1900). *Johns Hopkins Hosp. Repts.* **9**, 367.
Bijtel, J. H. (1958). *J. Embryol. Exptl. Morphol.* **6**, 466.
Butcher, E. O. (1933). *Am. J. Anat.* **53**, 177.
Byrnes, E. F. (1898). *J. Morphol.* **14**, 105.
Ciardi-Dupré, G. (1938). *Arch. ital. anat. embriol.* **41**, 1.
Couteaux, R. (1941). *Bull. biol. France et Belg.* **75**, 101.
Cuajunco, F. (1940). *Contribs. Embryol. Carnegie Inst.* **28**, 95.
Cuajunco, F. (1942). *Contribs. Embryol. Carnegie Inst.* **30**, 127.
Dalcq, A., and Pasteels, J. (1954). "Traité de Zoologie" (P. P. Grassé, ed.), Vol. 12. Masson, Paris.
Detwiler, S. R. (1934). *J. Exptl. Zool.* **67**, 395.
Detwiler, S. R. (1955). *J. Exptl. Zool* **129**, 45.
Duesberg, J (1909). *Arch. Zellforsch.* **4**, 602.
Filatow, D. (1933). *Wilhelm Roux' Arch. Entwicklungsmech. Organ.* **127**, 776.
Glucksmann, A. (1934). *Z. Anat Entwicklungsgeschichte* **103**, 303.
Glucksmann, A. (1951). *Biol Revs Cambridge Phil Soc.* **26**, 59.
Godlewski, E (1901). *Krakauer Anz.* **10**, 15.
Godlewski, E. (1902). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **60**, 1.
Graham Kerr, J (1919). "Textbook of Embryology," Vol. 2, Vertebrata. Macmillan, London
Haggqvist, G (1920). *Anat Anz* **52**, 389
Häggqvist, G. (1931). "Handbuch der mikroskopischen Anatomie des Menschen" (W von Mollendorff, ed), Vol 2, Pt 3. Springer, Berlin.
Haggqvist, G. (1956) "Handbuch der mikroskopischen Anatomie des Menschen" (W. Bargmann, ed), Vol. 2, Pt 4 Springer, Berlin
Hamburger, V. (1929) *Wilhelm Roux' Arch Entwicklungsmech. Organ* **119**, 47.
Hamburger, V. (1938). *J Exptl Zool* **77**, 379
Hamburger, V. (1939). *J Exptl Zool* **80**, 347
Harrison, R. O. G. (1904 *Am J Anat* **3**, 197.
Heidenhain, M. (1913). *Arch mikroskop Anat. u Entwicklungsmech.* **83**, 427.

His, W. (1868). "Untersuchungen über die erste Anlage des Wirbelthierleibes." Vogel, Leipzig.
Holtzer, H., Lash, J., and Holtzer, S. (1956). *Biol. Bull.* **111**, 303.
Huber, E. (1931). "Evolution of Facial Musculature and Facial Expression." Johns Hopkins Press, Baltimore, Maryland.
Hunter, R. McM. (1935). *J. Morphol.* **57**, 501.
Hunter, R. P. (1935). *J. Morphol.* **57**, 473.
Katznelson, Z. S. (1936). *Z. mikroskop.-anat. Forsch.* **40**, 474.
Lewis, W. H. (1910). *Anat. Record.* **4**, 183.
Lewis, W. H., and Lewis, M. (1917). *Am. J. Anat.* **22**, 169.
MacCallum, J. B. (1898). *Bull. Johns Hopkins Hosp.* **9**, 208.
McKenzie, J. (1955). *J. Anat.* **89**, 526.
Maurer, F. (1894). *Morphol. Jahrb.* **21**, 371.
Meves, F. (1907). *Anat. Anz.* **34**, 161.
Moscona, A. (1955). *Exptl. Cell. Research* **9**, 377.
Morpurgo, B. (1898). *Anat. Anz.* **15**, 200.
Muratori, G. (1939). *Anat. Anz. Erganzungsheft.* **87**, 430.
Mutchmore, W. (1951). *J. Exptl. Zool.* **118**, 137.
Prévost, –., and Lebert, –. (1844). *Ann. Sci. nat.* 3° série Zool. **1**, 193.
Remak, R. (1845). *Froriep's N. Notiz.* **55**, 57.
Rudnick, D. (1945). *J. Exptl. Zool.* **100**, 1.
Saunders, J. W. (1948a). *J. Exptl. Zool.* **108**, 363.
Saunders, J. W. (1948b). *Anat. Record* **100**, 756.
Schmidt, V. (1927). *Z. mikroskop-anat. Forsch.* **8**, 97.
Schwann, T. (1839). "Mikroskopische Untersuchungen über die Übereinstimmung in der Struktur und dem Wachstum der Tiere und Pflanzen." Berlin.
Smithberg, M. (1954). *J. Exptl. Zool.* **127**, 397.
Straus, W. L., and Rawles, M. E. (1953). *Am. J. Anat.* **92**, 471.
Streeter, G. L. (1942). *Contribs. Embryol. Carnegie Inst.* **30**, 231.
Streeter, G. L. (1949). *Contribs. Embryol. Carnegie Inst.* **33**, 151.
Strudel, G. (1955). *Arch. anat. microscop. et morphol. exptl.* **44**, 209.
Szepsenwol, J. (1947). *Anat. Record* **98**, 67.
Taylor, A. C. (1943). *Anat. Record* **87**, 379.
Tello, J. F. (1917). *Trabajos lab. invest. biol. univ. Madrid* **15**, 101.
Tello, J. F. (1922). *Z. Anat. Entwicklungsgeschichte* **64**, 348.
Theiler, K. (1957) *Acta Anat* **30**, 842.
Tschumi, P A. (1957) *J. Anat.* **91**, 149.
Weed, I. G. (1936) *Z. Zellforsch. u. mikroskop. Anat.* **25**, 516.
Wohlfart, G. [illegible] **1**[illegible]
Yamada, T. ([illegible] *rgan.* **137**, 151.
Yamada, T. (1939). *Okajimas Folia Anat Japon.* **18**, 565.

CHAPTER IV

Histochemistry of Developing Skeletal and Cardiac Muscle

E. B. BECKETT AND G. H. BOURNE

Although the morphological and histological study of embryonic development has been undertaken for many years now, little work has been carried out on the associated chemical aspects of differentiation, either from the biochemical or from the histochemical point of view.

Histochemical work, in particular, is of very recent date, and has mainly been concerned either with the development of the very early embryo (Brachet, 1940; Moog, 1944; Buño, 1951, 1954; Buño and Mariño, 1952), or with the processes involved in ossification (Horowitz, 1942; Lorch, 1947; Bevelander and Johnson, 1950; Pritchard, 1952).

A review of histochemical work on all fetal tissues was made by Rossi and associates (1954), and from this it was clear that very few data were available concerning any but the very earliest stages of embryonic development, and moreover, that information about developing muscle was almost nil. Since that time, as far as we are aware, the situation has remained much the same, and so most of the results to be described in this chapter will be those of the authors. For this reason, it may be well to emphasize that this chapter does not represent an authoritative survey of a comprehensive range of information, but is only a preliminary resume of the little which is as yet known.

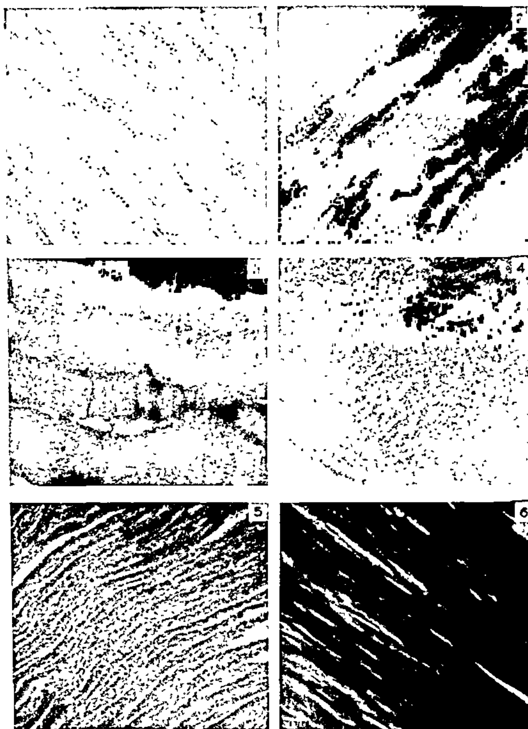


PLATE I

FIG. 1. Succinic dehydrogenase in skeletal muscle from a 10½ in. (100 day) fetus. The reaction is considerably weaker than in cardiac muscle.

I. SUCCINIC DEHYDROGENASE

An outline of the basic processes involved in the histochemical procedure for the demonstration of succinic dehydrogenase is given in Volume III, chapter IX on adult skeletal muscle. Exactly the same technique can be used for fetal tissues, but sometimes it is advantageous to use a higher pH (pH 8.2 instead of the more usual pH 7.5), since this enables regions of lower enzyme activity to be demonstrated (Rosa and Velardo, 1954). In fetal tissues there is less succinic dehydrogenase than in adult ones.

A. Cardiac Muscle

Succinic dehydrogenase activity in the hearts of developing chick and rat was studied biochemically by Sippel (1954). In the rat, it was found that there was a steep increase in enzyme activity between the 10th and 13th days, followed by a leveling off until the 16th day and then a further steep rise until the 19th day, i.e. 3 days before term.

Although histochemical observations do not readily lend themselves to quantitation, some interesting results have been obtained. Rossi and associates (1954) report that in human fetuses ranging in size from 60 mm. CR (crown-rump length) to 26 cm. CR, there is a strong reaction for succinic dehydrogenase. This has been confirmed by the present authors in hearts of 4-month-old human fetuses (Beckett and Bourne, 1958). In our series of goat fetuses, of 5¼ in. CR to 15 in. CR, the reaction intensity was moderate in the youngest fetus and progressively increased throughout prenatal life, although never attaining adult levels. In fetal cardiac musculature there was no evidence either of intercalated discs, which in the adult are negative (Bourne, 1953; Beckett and Bourne, 1958), or of Purkinje fibers, which

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Fig. 2. Succinic dehydrogenase in skeletal muscle of a 15 in. (133 day) fetus. Note the existence at this stage of fibers containing much and little enzyme activity.

Fig. 3. Succinic dehydrogenase in adult goat skeletal muscle.

Fig. 4. Succinic dehydrogenase in cardiac (ventricular) muscle from a 5¼ in (64 day) goat fetus.

Fig. 5. Succinic dehydrogenase in ventricular muscle from a 15 in (133 day) goat fetus.

Fig 6. Succinic dehydrogenase in adult goat ventricular muscle. Note the negative intercalated discs.

in the adult goat have a succinic dehydrogenase-positive nucleus and a positive reaction also in the peripheral myofibrils. Purkinje fibers are, however, morphologically differentiated by the 10½ in. CR stage in the goat, so that it seems that they must acquire their typical distribution of succinic dehydrogenase activity some time after differentiation.

B. Skeletal Muscle

Shen (1949), studying homogenates of fetal rat skeletal muscle, found a steadily increasing amount of succinic dehydrogenase activity present during the later part of fetal life. Rossi and associates (1954), on the other hand, claim that there is a strong reaction for succinic dehydrogenase in skeletal muscle at all stages of development of the human fetus. Our own observations are not in accord with those of Rossi and his colleagues, since in about a dozen specimens of skeletal muscle taken from three human fetuses of 16 weeks gestation we could find little succinic dehydrogenase activity. We also found that in goat fetuses the reaction intensity progresses from weak to moderate during prenatal life. In goat fetuses, little positive reaction was obtained until about two-thirds term (i.e. about 100 days). During the next 30 days or so there was a considerable increase in enzyme activity, so that by about 130 days the diformazan granules were arranged in a typical linear fashion, and in tibialis anterior, biceps, and gastrocnemius, it was possible to distinguish between the fibers containing little succinic dehydrogenase activity and those containing more of the enzyme. In these muscles there was no difference in size between the fibers showing different degrees of enzyme activity. In the rectus femoris, however, all of the muscle fibers showed equal reaction intensity.

Fig. 7. Acid phosphatase in the Golgi apparatus of Purkinje fibers of fetal goat cardiac muscle

Fig. 8. Acid phosphatase in the Golgi apparatus of Purkinje fibers of adult goat cardiac muscle.

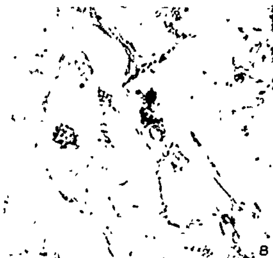
Fig. 9. Succinic dehydrogenase in Purkinje fibers in adult cardiac muscle. Note the positive reaction in the nucleus and at the periphery of each fiber.

Fig 10. Cholinesterase. Acetyl cholinesterase in end-plate areas in muscle from a 2½ in (48 day) goat fetus (L.P)

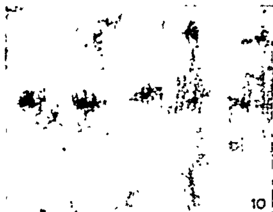
Fig. 11. High power view of acetyl cholinesterase in end-plate areas of muscle taken from a 2½ in. (48 day) goat fetus

Fig. 12 Acetyl cholinesterase in motor end-plates in muscle from a 5¼ in. (64 day) goat fetus.









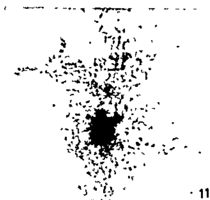




PLATE II

Despite the increase in succinic dehydrogenase activity during the last third of prenatal life, the degree of activity shown by fetal muscle was always considerably less than that exhibited by adult skeletal muscle. There was no evidence of any tendency for the diformazan granules to be more concentrated at the edges of the muscle fibers, although this distribution is often seen in adult human and goat skeletal muscle.

II. ESTERASES

The available information concerning the distribution of simple esterases and lipases in the fetal heart and in the skeletal muscle of fetuses can be very rapidly summarized. Buño and Mariño (1952) using Gomori's Tween technique, (see page 00 for details of method), obtained no reaction in the cells of cardiac and skeletal muscle of chick embryos of up to 18 days. Rossi and associates (1954) reported no esterase in the heart and blood vessels of the human fetus, and McKay *et al.* (1955) did not mention having obtained evidence of enzyme activity in cardiac or skeletal muscle fibers using the α-naphthyl acetate technique for esterases on an acetone-fixed 5 mm. human embryo, so one must assume that here too, these tissues were negative.

A. Cholinesterase in Motor End-Plate Areas

The chemical processes involved in histochemical techniques which demonstrate cholinesterase have been discussed elsewhere in this book (page 000). Although the distribution of cholinesterase has occasionally been studied in the adult heart (see for instance Gomori, 1948), as far as we are aware distribution of this enzyme in the embryonic heart has not yet been investigated, and so we must confine our remarks in this chapter to the distribution of cholinesterase in skeletal muscle of the fetus.

Kupfer and Koelle (1951), using the rat fetus as their subject, were the first to describe the development of cholinesterase in end-plate areas. They were unable to find cholinesterase in significant amounts, either histochemically, or biochemically, until the 16th day of gestation (term 21–22 days) At this time, the amount of enzyme present in the end-plate areas was low, since it required 50 minutes incubation to demonstrate it compared with the 10 minutes needed for adult muscle. The description of development of cholinesterase in the subneural apparatus given by these authors is confusing. Their preparations must

have shown the artifact of staining of muscle nuclei and the authors seemed to be under the impression that the subneural apparatus was produced by the fusion of bodies resembling nuclei, but which showed more cholinesterase activity than the rest of the muscle nuclei. However, the picture which emerges from their observations is of cholinesterase being present at poorly defined points on the muscle fibers at 16 days, followed by the development of the subneural apparatus during the next few days, until it is recognizable, at 20 days gestation, as being similar to that of the adult. After this time, structural development continues both in prenatal life and post partum.

The investigations of Kupfer and Koelle also demonstrated that there was no innervation of the end-plate areas until 21–22 days gestation, and it was suggested that cholinesterase might have a chemotactic effect on the nerve fibers. It is perhaps significant that East (1931) and Straus (1939) reported that the first movements of rat embryos in response to faradic stimuli occurred at 16 days. Opinions differ, however, about whether or not neuromuscular junction has occurred at this stage. Straus concurred with the view of Kupfer and Koelle that at this time no connection existed between nerve and muscle, whereas East thought that neuromuscular junction had just occurred. Both East and Straus considered that some change other than a morphological one was responsible for the appearance of contractility at the 16-day stage. Maybe the production of cholinesterase has something to do with this.

The pattern of the development of the subneural apparatus in the fetal goat is apparently totally different from that in the rat (Beckett and Bourne, 1958), unless the difference can be explained on technical grounds. In a 2½ in. CR goat fetus (i.e. about 48 days or one-third term), when the muscle fibers are at a very early stage of development, with few myofibrils and with centrally placed nuclei, there is a moderate amount of cholinesterase which is demonstrable after ¼ hour incubation. This cholinesterase is situated at points in rows running at right angles to the long axis of the muscle fibers, i.e., the distribution of these areas of activity is the same as that for end-plates in adult muscle. By the 64-day stage in the goat, cholinesterase activity in these areas has apparently risen to adult levels and this level is maintained for the rest of fetal life. This is in complete contrast to the slow development of enzyme activity in the rat.

When cholinesterase first appears in goat muscle fibers, there is

little in the way of structure to be seen in the areas of enzyme activity, but occasionally there is evidence of some sort of rudimentary subneural apparatus. This, instead of being in the complex pattern observed in the adult of many animals, is in the form of a short straight tube, or a crescent. At 64 days, the morphology of the subneural apparatus is reasonably clear. Some of these structures are still like a short tube or crescent, but others bear projections or outpocketings from the main tube. As prenatal life continues, the end-plate areas become larger and more complex. The original short tube or crescent seems to bend round to form a circle, and other tubular structures stretch from side to side of the circle in an irregular pattern, although the complexity and size seen in the end-plate of the adult goat are never attained before birth. In the adult goat the end-plates are larger and even more complex than those usually seen in the rat.

The subneural apparatus of human end-plates is present at 16 weeks and possibly earlier (Beckett and Bourne, 1958), since at 16 weeks the structure is like that of the goat at 64 days. The rate of development, however, must be slower than in the goat, since Coers (1955) found end-plates with a simple subneural apparatus in children of up to 1 year old, and he also observed that the adult character of end-plate structure emerged between 12–14 months of age. Similar observations have been made in other young animals by Gerebtzoff and co-workers (1954).

In a 6-month human fetus the enzyme in the end-plate area was found to be capable of splitting butyryl thiocholine as well as the acetyl compound (Beckett and Bourne, 1958) and it was eserine sensitive. Experiments to see if this was also true of other fetal human and goat muscle specimens were not carried out, but is it perhaps interesting that the only end-plates in the adult human which contain an enzyme that can split butyryl thiocholine are the "classic" end-plates, i.e., those similar in form to the ones described in other vertebrates. In human adult muscle, a wide variety of different morphological forms of end-plate exists, whereas in the fetus there is apparently only one form, i.e. the "classic" type. Whether all of the "classic" end-plates present in the fetus remain in that form until adult life and other structures present in the adult arise, *de novo*, after birth, or whether some of the "classic" end-plates in fetal muscle are converted to other forms later on, perhaps during childhood, cannot be said at the present moment, but it is a fascinating problem. Certainly by the age

of 9 years, there are a variety of different cholinesterase-positive structures in human muscle (Beckett and Bourne, 1957).

The work of the present authors cannot offer any evidence concerning the time at which junction between nerve and muscle occurs in the goat. Methylene blue preparations by Dr. Rubinstein of the London Hospital on one of the 16-week human fetuses of our series indicated the presence of rudimentary nerve endings, and as has been mentioned, the subneural apparatus of the 16-week human fetus is very like that of the 64-day goat fetus. No studies have been made of the neurological side of formation of motor end-plates in the goat, so that it is impossible to say whether or not cholinesterase is present before the nerves reach the end-plates and whether the enzyme might have a chemotactic effect on the growing nerve fibers. If it were known that neuromuscular junctions occurred at a definite stage of muscle fiber development, examination of hematoxylin-eosin preparations might have thrown some light on the problem, but is it obvious from the literature that the stage of development of the muscle fibers at which neuromuscular junction occurs is variable and probably depends on the species concerned. No work has been done on goat fetuses which correlates development of muscle fibers with the appearance of neuromuscular junctions, and so the question must remain unanswered for the time being.

B. Cholinesterase in Sites Other than Motor End-Plates

1. *Nerve Fibers*

In the course of the work of the present authors (Beckett and Bourne, 1958) cholinesterase-positive nerve axons were occasionally observed in fetal goat muscle and frequently in that of the adult of this species. In contrast to this, we have not so far detected cholinesterase in axons of human fetal muscle, and have very rarely seen it in nerves of adult human muscle. This apparent difference in enzyme activity of nerve fibers might be caused by differences in permeability of the myelin sheath to the reagent used to detect cholinesterase or it might be a true difference in levels of enzyme activity. It seems unlikely that the positive reaction seen in these structures is due to a backward diffusion of cholinesterase from the motor end-plates, since the enzyme activity is sometimes observed in nerve fibers situated some distance away from the motor end-plates, and moreover cholinesterase is not situated in the neural portion of end-plates.

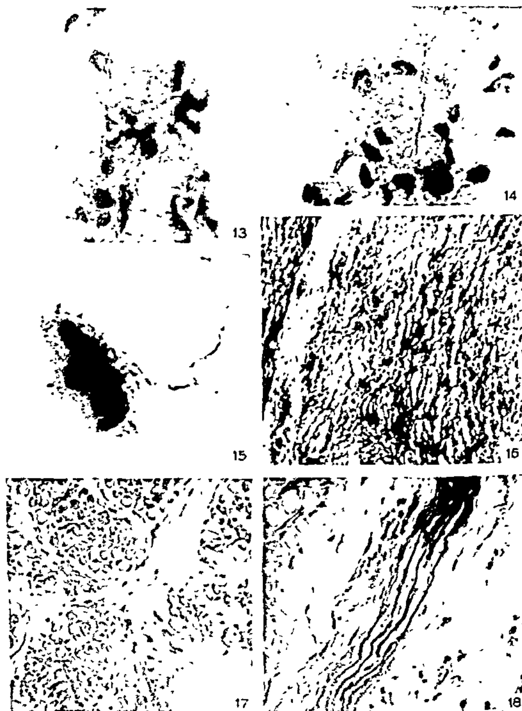


PLATE III

FIG. 13. Acetyl cholinesterase in end-plate areas in muscle from a 10 in. (100 day) goat fetus.

FIG. 14. Acetyl cholinesterase in motor end-plate areas of muscle from a 15 in. (133 day) goat fetus

2. Musculo-Tendinous Junctions

As has been mentioned elsewhere in this book (page 00), cholinesterase at musculo-tendinous junctions was first observed by Couteaux in 1953 in frogs, mice, and fish. Gerebtzoff and his colleagues have since demonstrated the same thing in a variety of other animals including man (Gerebtzoff, 1956), but the development of these cholinesterase-positive structures does not yet seem to have been described by authors other than ourselves.

In goat fetuses (Beckett and Bourne, 1958), structures at musculo-tendinous junctions make their appearance at about two-thirds term, (i.e. at about 100 days), which is considerably later than the time at which motor end-plates are first visible. At the 100-day stage the cholinesterase-positive material at musculo-tendinous junctions is in the form of small dots of structure similar to that of "gutters" of the subneural apparatus of end-plates and it contains little cholinesterase activity. During the time that follows, the musculo-tendinous junctions rapidly gain their adult form, i.e., the dots of "gutter" structure fuse together to form finger-like projections into the muscle substance, and they equally rapidly acquire their full complement of cholinesterase activity. For the remaining period of prenatal life, they increase in size but do not become more complex or apparently gain any more enzyme activity.

In the human fetus the structures at musculo-tendinous junctions are present in the early "dotted" form at 4 months, but at 6 months have changed very little in appearance, so that it seems that their development proceeds more slowly than in the goat.

III. PHOSPHATASES

A. Alkaline Phosphatase

The various histochemical methods for the demonstration of alkaline phosphatase have been described elsewhere (Volume III, Chapter IX). It seems that in most cases the Gomori-Takamatsu technique has been used for the study of fetal muscle, but just occasionally workers have used the α-naphthyl phosphate method (see for instance McKay *et al.*, 1955).

Fig. 15. Acetyl cholinesterase in a single motor end-plate from adult goat muscle. Note the enzyme activity in the nerve axon.

Fig. 16. 5-Nucleotidase in aortic wall of goat fetus.

Fig. 17. Acid phosphatase in skeletal muscle of fetus.

Fig. 18. Acid phosphatase in skeletal muscle of goat fetus showing positive axons.

1. Alkaline Phosphatase in Cardiac Muscle

The development of alkaline phosphatase of the heart appears to have been followed most closely in that of the human. McKay and his co-workers (1955) studied the heart of a 5 mm. human embryo, using both types of histochemical technique for the demonstration of alkaline phosphatase. They found that the auricular muscle gave a positive reaction with the Gomori technique, and that the walls of capillaries contained alkaline phosphatase which could be demonstrated by both methods. In addition, alkaline phosphatase could be demonstrated in the endothelium of the auricle by both techniques, while that of the ventricle gave a positive reaction only with the naphthyl phosphate method. The endothelium of the aorta also contained alkaline phosphatase.

Rossi and associates (1951) studied older human embryos (23 mm. CR to term), and during the next few years they extended their observations to the 9 mm. CR stage (Rossi *et al.*, 1954). They found that apart from a transitory alkaline phosphatase activity in the intima of the aorta and other large vessels in the 23 mm. CR embryo, this enzyme was restricted to the endothelium of the small vessels of the myocardium. Our own work, using the Gomori technique (Beckett and Bourne, 1958), showed that in the hearts of 3- and 4-month fetuses alkaline phosphatase activity was frequently to be found in the endothelial linings of the larger blood vessels of the myocardium and occasionally also in the endothelia of myocardial capillaries. There was, in addition, an occasional patchy reaction in the endocardium and in scattered areas of indifferentiated tissue. No activity was observed in any of the vessels entering or leaving the heart.

The development of alkaline phosphatase in the heart of the chick, which was studied histochemically with Gomori's technique by Moog (1944), appears to proceed in rather a different fashion from that in the human. At 72 hours, Moog obtained a positive reaction (after $1\frac{1}{2}$–4 hours incubation) in the endothelial lining of ventricle and atrium, and in the walls of the bulbus arteriosus, the ductus Cuvierii, and the ventral aorta. The cardinal veins were negative, and so also were the muscular walls of the atrium and ventricle. The nuclei of the myocardium showed transient activity only on the 4th day.

From the 4th to the 8th day of incubation, the nucleoli only of the myocardium were positive. The endothelial lining of the heart remained positive throughout this period, and so also did the linings

of the bulbus arteriosus and all vessels entering or leaving the heart. A high level of alkaline phosphatase activity was present in the septa, including the cushion septa.

The picture seen in fetal goat hearts appears to be very similar to that in the fetal human (Beckett and Bourne, 1958). At the 2½ in. CR stage (the earliest goat fetus studied), there was a reaction in the pericardium near the atrio-ventricular junction, but by the 5¼ in. CR stage this had disappeared. In the hearts of these early fetuses, there was alkaline phosphatase activity also in the endothelial linings of the myocardial blood vessels, but there were usually fewer positive vessels in the atria than in the ventricles. In older goat fetuses (up to term), only ventricular muscle was studied and here, as in the human hearts, most of the activity appeared to be centered in the endothelial linings of the vessels larger than capillary size. The number of positive capillaries was very variable. There was a slight tendency for more of the myocardial blood vessels to acquire alkaline phosphatase activity as prenatal life progressed but this tendency was ill defined, and one could not be certain of it because the number of vessels giving a positive reaction was so very variable at a given age.

The cardiac muscle of the adult goat gave no reaction at all under the incubation conditions which were used for the goat fetuses in the course of the work described above. This is perhaps interesting, since earlier work had indicated that in cardiac muscle of mouse, rat, and guinea pig (Zorzoli and Stowell, 1947) and of rat again (Bourne, 1953), the intercalated discs were alkaline phosphatase-positive. However, the different results may be the result of differing incubation periods, since for the work on goats 15–30 minutes was used, in contrast to the much longer periods used by Zorzoli and Stowell and by Bourne.

2. *Alkaline Phosphatase in Skeletal Muscle*

Although little work has been done on the subject of the distribution of alkaline phosphatase in embryonic skeletal muscle, it is fairly certain that its localization is essentially similar to that in both fetal cardiac muscle and adult skeletal muscle.

McKay *et al.* (1955) noted the presence of alkaline phosphatase in the fibrils of the myotomes of a 5 mm. human embryo, but in later fetuses it appeared that it was only the endothelial cells of blood vessels which contained this enzyme. Kabat and Furth (1941) observed a positive reaction in the capillary walls of embryonic mouse muscle,

while the present authors (Beckett and Bourne, 1958) found alkaline phosphatase in the endothelium of capillaries and of larger vessels in skeletal muscle of both goat and human fetuses. In the human fetus, however, the reaction in the intima of larger vessels was more common than that in the capillaries and there was slight alkaline phosphatase activity in the connective tissue between the striated muscle fibers. As in adult man, only a certain percentage of the total number of blood vessels of fetal muscle gave a positive alkaline phosphatase reaction. In fetal goats, this percentage and the total number of vessels which showed enzyme activity was variable in the extreme. There appears to be no correlation between the number of positive vessels and the age of the fetus from which the muscle was taken. In this, skeletal muscle closely resembled cardiac muscle.

In addition to the enzyme activity displayed by blood vessel walls, alkaline phosphatase could also be detected in nerve axons of fetal goat muscle (Beckett and Bourne, 1958). No positive nerve axons were, however, found in muscle of 3- and 4-month human fetuses. Alkaline phosphatase activity had been previously noted in developing nerve fibers of Amblystoma embryos by Elftman and Copenhaver (1947) and in the peripheral nerves of a 5 mm. human embryo by McKay *et al.* (1955).

B. Acid Phosphatase

Acid phosphatase, as demonstrated by the Gomori lead phosphate technique (Volume III, Chapter IX), has a much more widespread distribution than alkaline phosphatase, although, like this latter enzyme, it has been little studied in fetal striated musculature.

1. *Acid Phosphatase in Cardiac Muscle*

a. Cardiac Blood Vessels. Rossi *et al.* (1952), reporting their findings on human fetuses of from 1 to 7 months gestation, stated that no acid phosphatase activity could be detected in blood vessels of any type even after 20–48 hours incubation with substrate. Later work by these authors showed that there was no acid phosphatase in the major blood vessels of the heart of a 9 mm. CR human embryo, and that at later stages of development (up to 36 cm. CH), some of the large blood vessels were negative, while others showed a positive reaction in their mesenchymal nuclei.

The results described by Rossi and his co-workers are in complete

contrast to those obtained by the present authors (Beckett and Bourne, 1958) using intact hearts of $2^1/_2$–$5^1/_4$ in. CR goat fetuses and of 12 to 16 week human fetuses, in addition to specimens of ventricular muscle taken from goat fetuses of from 10 to 18 in. CR. Our incubation periods for the demonstration of acid phosphatase were 4–6 hours only.

In fetal goat hearts, the strongest reaction was in the walls of all blood vessels except capillaries. The reaction was located both in the fibroblasts and in the nuclei of all types of cell present in the blood vessel walls. The picture seen in the human fetal hearts was essentially the same, but in the human the most intense reaction was situated in cone-shaped groups of granules lying at the poles of the nuclei of the great vessels entering or leaving the heart. In addition, the nucleoli of blood vessels (which are always more strongly positive than the nuclei of these structures) were more conspicuous in human than in goat hearts.

There was a second striking difference between goat and human to be observed in these intact hearts. There were present beneath the endocardium of the human hearts, particularly in the atrial region, some large cells, squarish in shape and packed with large granules which gave a most intense acid phosphatase reaction. These cells looked very much like mast cells, but could not be stained either metachromatically, or with Gomori's (1950) aldehyde fuchsin, so that this morphological identification is likely to be incorrect.

b. Purkinje fibers. The only observations on acid phosphatase in Purkinje fibers seem to be those of the present authors. This is perhaps not surprising, since most other work has made use of human fetal material, and we could find no trace of these fibers in the human fetal hearts of our series. In fetal goat hearts, a strikingly intense acid phosphatase reaction was present in the perinuclear zone of Purkinje fibers, i.e., in what probably represents the Golgi apparatus of these fibers. This very intense reaction was found in all specimens, except one of $2^1/_2$ in. CR and one of 18 in. CR, and was also very clearly demonstrable in adult goat heart. The nucleus and peripheral myofibrils of the Purkinje fibers gave a light to moderate reaction intensity.

c. Myocardium. Rossi *et al.* (1952, 1953) reported that in their series of human fetuses, which ranged from 9 mm. CR to 36 cm. CH, the reaction in the myocardium was feeble and was limited to the nuclei of the muscle fibers. Our own results (Beckett and Bourne, 1958)

showed rather a different picture. The reaction intensity was very variable, and there was no observable correlation with the age of the fetus concerned, or with any known technical variation. One must therefore reserve judgment about whether the different reaction intensities reflected genuine variations in the level of enzyme activity or whether they were due to lack of reliability of the technique. In spite of the difficulties of interpretation arising from the variability of reaction intensity, something can be said about the distribution of acid phosphatase activity.

In general, in the fetal goat, the greatest reaction intensity was observed in the nucleoli of the myocardium. There was rather less activity in the nuclei, and a light to moderate reaction for acid phosphatase in the cytoplasm. Where the cytoplasmic activity rose to moderate levels, cross striations were visible, and in the adult, the intercalated discs were enzyme-positive. There appeared to be no significant difference in enzyme activity between auricular and ventricular muscle, in either the young fetal goat hearts or the human hearts.

In addition to the sites previously mentioned, acid phosphatase was sometimes also present in groups of granules at the poles of the nuclei. These granules have in the past been designated either as the Golgi apparatus (Beams, 1929; MacDougald, 1936; Eastlick, 1937; Moog, 1944) or as perinuclear sarcosomes (Kisch, 1951; Cleland and Slater 1953). They were strongly positive in all four human hearts which were examined, but they were not a constant feature of the fetal goat cardiac muscle. In this latter tissue there were no enzyme-positive perinuclear granules at the 2½ in. CR stage, but they made their first appearance at 5¼ in. CR (64 days). After this, acid phosphatase activity in the granules increased, reaching a maximum at about 100–110 days, and then decreased again, reaching zero at 120-130 days, and staying at this level until term (150 days). As, however, this sequence has been observed only in one series of 21 specimens, one cannot be certain whether or not it represents a true picture of events. It will be necessary to make many further observations before this apparent rise and fall in acid phosphatase activity can be accepted as being normal part of embryonic development. If, indeed, the acid phosphatase activity in these perinuclear granules does fall away to zero during the last part of fetal life, it must be regained at some later stage, since the cardiac muscle of the adult goat again shows these enzyme-positive granules.

2. Acid Phosphatase in Skeletal Muscle

As in the case of fetal cardiac muscle, very few observations have been made on the distribution of acid phosphatase in fetal skeletal muscle, but the pattern of enzyme activity appears to be very similar in the two tissues, and very similar also to that observed in adult skeletal muscle.

In the chick, there appears to be little acid phosphatase in muscle except in the very early stages of development. Moog (1944) reported that this tissue lost practically all its acid phosphatase activity during differentiation from the mesoderm. Rossi *et al.* (1953, 1954) found a similar picture in fetal human muscle, since they were able to obtain a positive reaction only in the nuclei of the skeletal muscle of their series of fetuses.

In contrast to the observations mentioned above, Wolf *et al.* (1943) found quite a lot of acid phosphatase activity in adult skeletal muscle of various animals. In the skeletal muscle fibers themselves, there was a moderately intense reaction in the nuclei and sarcolemma, and the cross striations were sometimes visible. In addition, the smooth muscle cells and the nuclei of all cell types in the walls of arteries and veins contained acid phosphatase. There was also a strong reaction for this enzyme in the axons, the nuclei, and cytoplasm of Schwann cells and in the endoneurial cells of peripheral nerves present in skeletal muscle.

The observations of the present authors (Beckett and Bourne, 1958), on fetal goat and fetal human muscle are much more in tune with those of Wolf *et al.* (1943) than with those of Rossi *et al.* (1953, 1954) or Moog (1944). The over-all reaction intensity observed in the course of our work on skeletal muscle was, as with cardiac muscle, variable in the extreme, and was apparently not correlated either with fetal age or with known technical differences. A high reaction intensity was seen in nerve axons, and in the perinuclear granules of both the muscle fibers themselves and of the connective tissue cells. There was a large variation in the number of groups of positive perinuclear granules. The variation appeared to be random and not connected with different stages of prenatal life. In fetal human muscle, the perinuclear granules were far less conspicous than those in fetal goat muscle. The nucleoli of all structures contained in skeletal muscle of both fetal goat and fetal human possessed a high degree of acid phosphatase activity. In addition, a moderate reaction was observed in the walls of blood vessels, and in the nuclei of both connective tissue and skeletal muscle fibers.

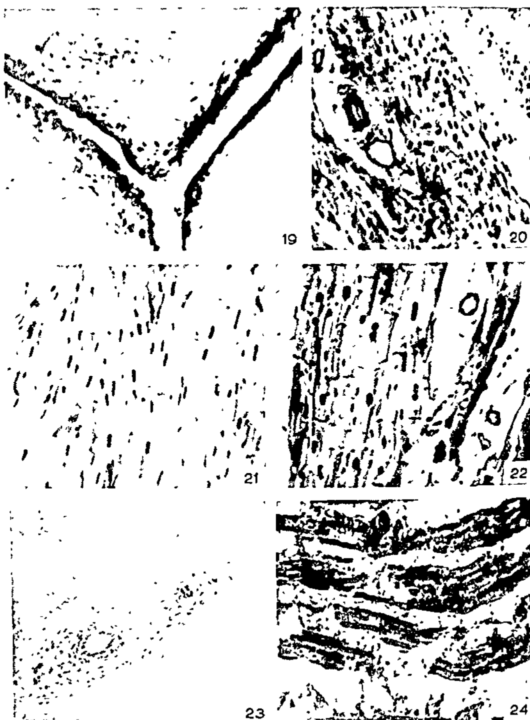


PLATE IV

FIG. 19. 5-Nucleotidase A weak reaction in fetal goat cardiac muscle. The enzyme activity is limited to the endothelial lining of the heart

FIG. 20. 5-Nucleotidase A strong reaction in fetal goat cardiac muscle. Enzyme

Sometimes acid phosphatase was present in the muscle fibers themselves, and cross striations were then positive. As with the perinuclear granules, the variation in acid phosphatase activity in the muscle fiber seemed to be completely random.

IV. 5-NUCLEOTIDASE

The technique for the demonstration of this enzyme in acetone-fixed material was introduced by Gomori (page 000), but it has been very rarely used for the study of fetal muscle.

A. 5-Nucleotidase in Cardiac Muscle

McKay *et al.* (1955) noticed that 5-nucleotidase was present in the endothelium of the heart of a 5 mm. human embryo. The work of the present authors (Beckett and Bourne, 1958) has to some extent confirmed that of McKay *et al.* and has amplified it. Our observations showed that in fetal muscle there was a specific enzyme which would hydrolyze 5-nucleotide, but not glycerophosphate, at pH 8.25, and which had a distribution totally different from alkaline phosphatase. Long incubation periods (8 or 16 hours) were required for the demonstration of 5-nucleotidase, but its localization in fetal muscle was precise, with no evidence of diffusion. It must be pointed out, however, that in some other tissues, for instance rat muscle, the incubation period must be considerably shortened in order to attain clear cut localization of enzyme activity.

After 8 hours incubation, the hearts of the 2½ in. CR goat fetuses used in our series showed 5-nucleotidase activity in the endocardium and in auricular muscle. The intimas of the larger blood vessels (i.e. those larger than capillaries) and the walls of the aorta, where this structure

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activity is present in the cardiac muscle fibers themselves as well as in the walls of blood vessels, etc.

Fig. 21. 5-Nucleotidase. Enzyme activity in adult goat cardiac muscle. Note the positive nuclei and intercalated discs.

Fig. 22. 5-Nucleotidase. Enzyme activity in adult human cardiac muscle. The distribution is similar to that seen in the goat. (The technique was carried out at pH 7.8 for this tissue.)

Fig. 23. 5-Nucleotidase. A weak reaction in fetal goat skeletal muscle. Enzyme activity is limited to blood vessel walls.

Fig. 24. 5-Nucleotidase. A strong reaction in fetal goat skeletal muscle. There is a great deal of activity in the muscle fibers themselves as well as in blood vessel walls and nerve fibers.

was present in the section, also gave a positive reaction. In all of these sites there was high enzyme activity in the nuclei and moderate activity in the cytoplasm. There was no 5-nucleotidase present in ventricular muscle. If the incubation period was extended to 16 hours, the reaction intensity became greater and the nuclei of the ventricular muscle adjacent to the endocardium appeared to contain 5-nucleotidase. Whether the positive reaction in ventricular nuclei was due to diffusion or to the existence of 5-nucleotidase in them is difficult to decide.

In the 3-month human fetal heart, after 8 hours incubation with substrate, 5-nucleotidase activity was almost entirely located in the endocardium and a little of the underlying tissue, and also in the heart valves. As in goat, the reaction was primarily nuclear and there was no activity in ventricular muscle. After longer periods of incubation, 5-nucleotidase activity was visible in the nuclei of the walls of the smaller blood vessels and in auricular muscle.

The degree of enzyme activity displayed in specimens of ventricular muscle taken from older goat fetuses was very variable. In the sarcoplasm and myofibrils of both cardiac muscle fibers and Purkinje fibers, the reaction intensity varied from nil to very intense. Most 5-nucleotidase activity was observed in the endocardium and in the nuclei and intimas of the arteries and veins of the myocardium. In addition, there was much enzyme activity in the nuclei of both capillaries and loose connective tissue, and also in the nucleoli of the cardiac muscle fibers. Both adult goat cardiac muscle and that obtained from an 18 in. CR (term) fetus, showed a slight reaction in the sarcoplasm and myofibrils, and a strong positive reaction in intercalated discs. A similar distribution was seen in human adult cardiac muscle, but with one difference. The nucleoli of the latter tissue contain less 5-nucleotidase activity compared with their nuclei than do the nucleoli of goat cardiac muscle nuclei. The nuclei are therefore more conspicuous in the goat.

B. 5-Nucleotidase in Skeletal Muscle

For the discussion of this subject, the authors will again have to rely only on their own observations on goat and human fetal muscle, since there appears to be no reference to other work in the literature. The distribution of 5-nucleotidase in skeletal muscle was very similar to that in cardiac muscle and was equally variable. Most 5-nucleotidase

activity in fetal muscle was present both in connective tissue fibers and nuclei of the arteries and veins. Equally high enzyme activity was present in tendon fibroblasts and in the nuclei and fibers of connective tissue situated between the muscle fibers. In addition, in the goat, but not in the human, there was a strong reaction in the axons and connective tissue sheaths of nerves present.

The over-all reaction intensity in fetal goat muscle was extremely variable, and this was reflected in the extent to which the muscle fibers themselves displayed enzyme activity. Although in muscle fibers enzyme activity varied from nil to very intense, there appeared to be no correlation between the reaction intensity and the age of the fetus from which the muscle was taken. Adult goat and human muscle showed either no 5-nucleotidase activity at all, or a reaction which was limited to the walls of blood vessels of larger size than capillaries.

V. SULFHYDRYL AND AMINO GROUPS

The histochemical procedures for demonstration of these two types of protein-bound groupings (Volume III, Chapter IX) were carried out by the present authors on one or two specimens of fetal goat and human skeletal muscle. Although the number of specimens used was so small that the results can only be regarded as tentative, it may be worth while recording that there appeared to be a consistently lower concentration of these groups in fetal than in adult muscle.

VI. SUMMARY

1. Succinic dehydrogenase activity in fetal cardiac muscle develops from moderate to strong during the course of prenatal life, but never reaches levels seen in adult muscle. In the adult, the intercalated discs are negative and the Purkinje fibers show a positive reaction in their nuclei and in their peripheral myofibrils.

2. The level of succinic dehydrogenase activity in skeletal muscle is considerably lower than that observed in cardiac muscle, and it increases during fetal life. The difference between fibers having a low succinic dehydrogenase activity and those having a higher activity becomes apparent before birth.

3. There is probably no simple esterase or lipase activity in fetal cardiac or skeletal muscle.

4. In the rat, cholinesterase does not appear in end-plate areas in appreciable amounts until 16 days (21-22 days is full term), whereas

in the goat and human it is present much earlier, and in fact is there by the time that about one-third of the total prenatal life has passed. It is probable that the development of the subneural apparatus takes place much more rapidly in the goat than in the human after the first appearance of cholinesterase activity.

5. In the goat, cholinesterase at musculo-tendinous junctions appears later than that in end-plate areas. This enzyme is sometimes also *present in nerve axons in the fetal goat, but not in the fetal human.*

6. Except at very early stages of development, alkaline phosphatase activity is almost entirely limited to the endothelium of capillaries and larger blood vessels in both cardiac and skeletal muscle.

7. Acid phosphatase has a more widespread distribution than the phosphatase demonstrable at alkaline pH. In both cardiac and skeletal muscle, most activity is centered on the nucleoli and perinuclear granules. There is rather less activity in nuclei, and less still in the striated muscle fibers themselves and the smooth muscle cells of blood vessels. The over-all reaction intensity of this acid phosphatase is extremely variable, but it is not certain that this is due to technical difficulties. Acid phosphatase activity is sometimes present in peripheral nerves of skeletal muscle.

8. There appears to be a specific enzyme in cardiac and skeletal muscle which is capable of hydrolyzing 5-nucleotide. This enzyme is particularly associated with nuclei of all types and with membranes. It is therefore limited in distribution to the walls of blood vessels of larger caliber than capillaries and to the endocardium when there is little total activity. When there is more activity present, it is also situated in the nuclei of connective tissue and sometimes in the substance of muscle fibers. On the whole, there is more 5-nucleotidase in fetal than in adult striated muscle.

References

Beams, H W (1929) *Anat. Record* **44**, 237.
Beckett, E B., and Bourne, G H. (1957). *J Neurol Neurosurg. Psychiat.* **20**, 191.
Beckett, E. B , and Bourne, G H. (1958). *Acta Anat.* **35**, 224.
Bevelander, G , and Johnson, P L (1950). *Anat. Record* **108**, 1.
Bourne, G H (1953) *Nature* **172**, 588
Brachet, J. (1940). *Arch biol. (Liége)* **51**, 167.
Buño, W. (1951). *Anat Record* **111**, 123.
Buño, W. (1954) *Gaz Méd Portuguesa* **VII**, 198
Buño, W and Mariño, R G (1952). *Acta Anat.* **16**, 85.
Cleland, E C , and Slater, K W (1953) *Quart J. Microscop. Sci.* **94**, 329.

Coërs, C. (1955). *Acta Neurol. Psychiat. Belg.* **55**, 741.
East, E. W. (1931). *Anat. Record* **50**, 201.
Eastlick, H. L. (1937). *J. Morphol.* **61**, 399.
Elftman, H., and Copenhaver, W. M. (1947). *Anat. Record* **97**, 385.
Gerebtzoff, M. A. (1956). *Extraits Ann. Histochim.* **1**, 26.
Gerebtzoff, M. A., Philippot, E., and Dallemagne, M. J. (1954). *Acta Anat.* **20**, 237.
Gomori, G. (1948). *Proc. Soc. Exptl. Biol. Med.* **68**, 354.
Gomori, G. (1950). *Am. J. Clin. Pathol.* **20**, 665.
Horowitz, N. H. (1942). *J. Dental Research* **22**, 519.
Kabat, E. A., and Furth, J. (1941). *Am. J. Pathol.* **17**, 303.
Kisch, B. (1951). *Exptl. Med. Surg.* **9**, 333.
Kupfer, C., and Koelle, G. (1951). *J. Exptl. Zool.* **116**, 397.
Lorch, I. J. (1947). *Quart J. Microscop. Sci.* **88**, 67.
MacDougald, T. J. (1936). *Z. Zellforsch. u. mikroskop. Anat.* **24**, 399.
McKay, D. G., Adams, E. C., Hertig, A. T., and Danziger, S. (1955). *Anat. Record* **122**, 125.
Moog F. (1944). *Biol. Bull.* **86**, 51.
Pritchard, J. J. (1952). *J. Anat.* **86**, 259.
Rosa, C. G., and Velardo, J. T. (1954). *J. Histochem. and Cytochem.* **2**, 110.
Rossi, F., Pescetto, G., and Reale, E. (1951). *Z. Anat. Entwicklungsgeschichte* **115**, 500.
Rossi, F., Pescetto, G., and Reale, E. (1952). *Ext. compt. rend. de l'Assoc. des Anat. 39th Re-union*, 744.
Rossi, F., Pescetto, G., and Reale, E. (1953). *Z. Anat. Entwicklungsgeschichte* **117**, 36.
Rossi, F., Reale, E., and Pescetto, G. (1954). *Ext. compt. rend. de L'Assoc. des Anat.* p.p. 1—102.
Shen, S. C. (1949). *Anat. Record* **105**, 489.
Sippel, T. O. (1954). *J. Exptl. Zool.* **126**, 205.
Straus, W. L. (1939). *Anat. Record* **3**, *Suppl.* **109**, 50.
Wolf, A., Kabat, E. A., and Newman, W. (1943). *Am. J. Pathol.* **19**, 423.
Zorzoli, A., and Stowell, R. E. (1947). *Anat. Record* **97**, 495.

CHAPTER V

Skeletal Muscle Tissue in Culture

MARGARET R. MURRAY

I. INTRODUCTION

From the earliest days of tissue culture, muscle has attracted the attention of investigators, who lost no time in examining the *in vitro* behavior of the major tissue types. Against a background of movement where tissues normally fixed (or "noble") took on a plastic and migratory habit, all three kinds of muscle were conspicuous by their demonstrated ability to contract autonomously and rhythmically in isolation from the nervous and other controls exerted by the whole organism. In the Lewises' culture medium (which consisted of Locke solution plus 0.5% dextrose and 10% chicken bouillon), contraction was especially rife; it is sobering to read the early papers on muscle by these superlative observers (Lewis and Lewis, 1917; M.R. Lewis, 1920) and to reflect on how little proportionately has been added since by a legion of busy researchers. Problems of development and of structure in relation to function occupied the early workers; for these workers, tissue culture seemed to have been especially designed, since it threw open the living somatic cell to microscopic observation—serially and under conditions of experiment. The *in vitro* observations of Burrows (1912), Lake (1915), and others contributed substantially to the myogenic theory of heart action: it was early shown also that skeletal muscle is intrinsically capable of rhythmic contraction in the total

absence of innervation (M.R. Lewis, 1915), and that the striated types of muscle operate generically like smooth muscle in being able to contract before any visible evidence of cross striation exists (reconfirmed by Szepsenwol, 1946 and Holtzer *et al.*, 1959). Much of this early work was reviewed and discussed by Levi in his monograph of 1934, and will not be elaborated here. Areas will be selected, however, in which tissue culture actually or potentially offers some special insight into muscle problems through its ability to separate the parts of a system and maintain them in health and isolation.

II. HISTOGENESIS

A. Induction and Cytodifferentiation

In normal development *in vivo*, muscle differentiation is probably brought about by the synergistic action of various neighboring tissues. From grafting experiments, it appears that not only the somite, but also the pronephros area of the neurula, is capable of forming musculature in a suitable environment. Differentiation of the trunk mesoderm is envisaged by Muchmore (1951) in terms of several overlapping morphogenetic fields which are characterized by qualitative rather than quantitative differences; the notochord has been repeatedly implicated as an inducing factor in the determination of skeletal muscle.

There is no doubt that mesodermal areas of the chick destined to become muscle are capable of autonomy when isolated *in vitro* at a stage preceding histological differentiation. Explanted at 3–4 days, the musculature of the limb bud, as shown by van Weel (1948), is able to develop and maintain a characteristically high content of ascorbic acid; glycogen appears in these cells later in the course of cultivation when they are differentiated into muscle fibers. In the limb bud isolated at this period, skeletal primordia are present, though neural and chordal influences are eliminated. Recently, Avery *et al.* (1956) have cultured somites of stages 14–24 alone and in combination with notochord or spinal cord. They found no evidence that the presence of notochord in early stages enhances muscle development; at stage 24, somites alone will differentiate into muscle fibers. The presence of spinal cord appears to accelerate the growth of somitic muscle. In isolated somites, the capacity to develop cartilage precedes the capacity to develop muscle, appearing at stage 20.

A fruitful approach to problems of tissue interaction is afforded by

the method of cell dissociation, recently extended by Moscona (1956) to effect development *in vitro* of heterotypic combinations of embryonic cells. Using chick embryos from stage 16 to 5 days, he found that in the presence of chondrogenic cells myoblasts did not readily attain advanced differentiation unless they were present in abnormal amounts. Even when tissue suspensions were made from a 4-day limb bud, no myogenic cells differentiated, though dissociated chondrogenic cells proceeded to reaggregate and form typical cartilage. In mixed aggregates, myogenic cells, if present in large excess, formed the outer sheath of the cluster with cartilage restricted to the interior. Moscona here discusses the principle of "liminal proportional concentration," suggesting that the characteristic histogenic activity of cells of a given type may be related to their share in the total population, and may be suppressed when this is below a given level. Factors which may enter into the making of the total effect of population density are CO_2–O_2 ratio, shifts in water balance, and even the mechanical nature of the substrate which if consisting of submicroscopical fibrils (as in bacterial cellulose, cellophane, or reconstituted collagen) allows the cells to become firmly attached *in situ* instead of wandering off.

In an account of earliest changes in structure and behavior of differentiating amphibian cells, Holtfreter (1947) notes that skeletal myoblasts isolated from the gastrula or from older forms have an inherent polarity. They adopt a plump spindle shape or a nonadhesive cylindrical modification of this, and then stretch out into a long spindle with pseudopods at each end but with a hyaline end-plate to distinguish the anterior pole. Elongation results from successive forward movements of the undulating end-plate and a passive stretching and spinning out of the central cell portion, which is not adhesive to glass. He finds no tendency of myoblasts to adhere in a group and therefore concludes that the parallel orientation of fibers in a somite is not due to tactoid forces or to any molecular zipper mechanism, but rather to the original parallelism of the cell axes and to the spatial conditions of growth as supplied e.g. by the mesenchyme matrix.

Returning to the chick, Moscona (1958) has observed discrete myogenic cells dissociated from 4-day limb buds assume polygonal shapes typical of fibroblasts. The myoblasts, however, contained characteristic granular inclusions of low polysaccharide and high nucleoprotein content, which were the first visual indication of differentiation. From this point, the cells underwent mitotic divisions

and then elongated to take on axial structure, losing the granules and developing in their stead longitudinal strands of nonstriated proto-myofibrils. Such cells responded to mechanical stimulation with a twitch. These myoblasts continued to elongate, becoming multi-nucleate. Cross-striations usually did not become visible in fibrils until the cell had reached the sarcoblast stage, but occasionally they were noted in spindle-shaped, mononucleate cells. It is not known whether the appearance of banding as seen by the light microscope represents a change in the constitution of the myofibrils, or whether it is due to their becoming appropriately arranged, aligned, and fasciculated.

Using explants from 7–11 day chick embryos, in which sarcoblasts were already formed and in some cases cross-striated, Lewis and Lewis (1917) observed "muscle buds" growing out from old fibers. These elongated structures were less differentiated than the parent sarcoblast; they did not develop cross-banding as a rule in the short period of cultivation (about 5 days) used by these workers. Where striations occurred, they occupied only a small portion of the bud, not directly continuous with the old striated fiber, and were not well marked. Fixation brought to light some banding that was not visible in life, in myoblasts as well as in sarcoblasts. Longitudinal striations were usual, but these were not regarded as myofibrils but as tension artifacts, surface folds emphasized by fixation. W. H. Lewis (1923) in dark-field examination of all three types of muscle *in vitro*, observed no intra-cytoplasmic fibrils.

B. Fibrogenesis and Nuclear Division

The Lewises and subsequent workers (Levi, 1934) with embryonic tissues have described two main out-growing or out-wandering cell types: the bud or sarcoblastic prolongation of a part of an already constituted fiber, and the spindle shaped myoblast. It is accepted by all that the early myoblast can and frequently does undergo mitotic division; agreement has not been reached on the mode of formation of the multinucleate sarcoblast. Though sarcoblasts may rapidly increase in length and in number of contained nuclei, mitotic figures are rarely observed in them. Are these multinucleate structures the result of amitotic division or fragmentation of the large vesicular nuclei originally present in young elongating cells, or are they the product of fusion of many myoblasts? (Plate I, Fig. 1.)

Lewis and Lewis (1917), unlike Holtfreter, noted a marked

tendency for anastomosis and fusion between separate elongating muscle buds, following chance contact; branches containing one, two, or several nuclei might also split off from sarcoblasts and wander freely on glass. Some buds seemed to consist of chains of myoblasts which tended to break apart from one another. The authors observed neither mitosis nor amitosis in multinucleate sarcoblasts, but questioned whether the apparent amitotic stages seen in fixed preparations represented the typical mode of nuclear multiplication. Levi (1934) believed their skepticism to be unwarranted. Recently, Rinaldini (1957), and Wilde (1958), working independently at the Strangeways Laboratory, have reported verbally on observations of myoblast coalescence. Wilde in particular has observed fusion of myoblasts from chick and mouse, to form contractile fibers containing longitudinal fibrils.

Moscona (1958) has observed cleaving nuclei, otherwise in interphase configuration, and as many as three furrows dividing a nucleus into sectors, each with its nucleolus. Chèvremont (1940) described amitosis in skeletal muscle cultivated *in vitro*; Frédéric (1954), in an experimental study of chick myoblasts utilizing time lapse motion pictures, observed three direct divisions, although the more usual mode of division is by mitosis (40 cases) for cells in this stage of development. Frédéric observed in one cell that the nucleus first divided amitotically; the daughter nuclei then fused again and the cell went on to divide normally by mitosis. Pogogeff and Murray (1946) have observed suggestive configurations in the large vesicular nuclei of elongating myoblasts; no mitoses were seen. Godman and Murray (1953), administering colchicine to developing sarcoblasts, saw no arrested mitoses. Godman (1957) again reports that effective doses of colchicine are without influence on the appearance or numbers of sarcoblastic nuclei. According to I. R. Konigsberg (1958), treatment of cultures with nitrogen mustard (which exerts a preferential effect on DNA synthesis) does not prevent the massive formation of multinucleate sarcoblasts. At high concentrations, the lethal effects of this alkylating agent seem to be exerted preferentially on mononuclear cells.

To the writer, it appears that the above views of sarcoblast formation need not be conflicting, and are not mutually exclusive. Coalescence of fibers and of myoblasts has been observed repeatedly. The Lewises' and later observations that fibers might disintegrate into component myoblasts suggest that this union is not necessarily permanent.

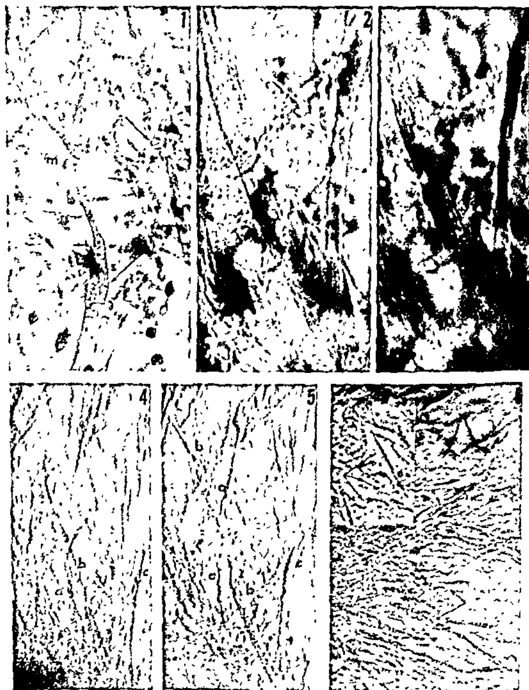


PLATE I

FIG. 1. Embryonic muscle, 18-day rat fetus, 3 days *in vitro* Zenker's fixative, Delafield's hematoxylin stain Magnification: × 150. Note nuclear furrows and crowding, also differences in nuclear size (arrows), suggestive evidence for amitosis. *M*, myoblasts; *S*, sarcoblast.

FIGS. 2 and 3. Living adult human muscle, 20 and 21 days *in vitro*. Note longitudinal fibrils Arrow points to region of apparent fusion between two sarcoblasts. Magnification: × 300.

Regeneration studies to be cited later indicate that fully differentiated fibers also may break up into viable mononucleate forms which are capable of redifferentiation. The paucity of mitoses during intermediate and late myodifferentiation and the concomitant increase in mass of protoplasm and number of nuclei point to direct division as an effective multiplication method. This is supported by the drug experiments and by actual observation in cases. It seems probable that the normal course of fiber development may involve both of these processes—myoblast fusion and subsequent direct nuclear division—in varying degrees (Plate I, Fig. 1–5; Plate II, Figs. 8 and 11).

III. MORPHOLOGY

A. Fiber Structure

1. *Microdissection*

Microdissection studies by Chambers and Fell (1931–1932) and by de Rényi and Hogue (1934) showed that the sarcolemma of the living myoblast and sarcoblast is a structureless hyaline membrane of great toughness and unusual thickness; it is fairly rigid, and does not disintegrate for some minutes after injury, unlike membranes of fibroblasts, which are much less substantial. It is always present on cultured muscle cells, and is not formed by the connective tissue component of muscles. When the sarcolemma is torn, the sarcoplasm of these non-striated cells wells out as a jellied mass, which is denser and more refractile than the protoplasm of fibroblasts. The sarcoplasm in resting fibers is more plastic and less solid than in fibers undergoing contraction. The apparent fusion of fibers does not necessarily produce actually confluent cytoplasm, as shown by micro-injection tests. The substantial sarcolemma must offer a considerable barrier to fusion. Mechanical stimulation of a long fiber may result in contraction of only the detached end of the sarcoblast. This can gradually become re-extended; but alternatively may break up into many myoblasts. Such a process would require some solation of the sarcolemma.

Figs 4 and 5. Living adult human muscle 27 and 30 days *in vitro*. Separation of distal tips and migration away from parent fibers *a* and *b*. Fiber *c* shows the beginning of a [illegible]

Fig. [illegible] culture of adult human muscle, [illegible] × 200.

Photomicrographs and observations are by Pogogeff and Murray.



PLATE II

FIG. 7. 8-Day culture, showing cross-banding in fibrillar arrangement, on each side of the nucleus. A juxta-nuclear area is occupied by clear vacuoles. Magnification: × 1500.

FIG 8. Branching or fusing sarcoblasts with irregular banding; fatty vacuoles 9-day culture. Magnification × 1600

FIG 9. Sarcoblast of 9-day culture with highly developed, comparatively regular banding. Arrows point to M bands. Magnification· × 1600.

2. *Colchicine Response*

The remarkable disrupting effect upon striated muscle ribbons of colchicine in high dilution (up to 10^{-4} *M*) is described by Godman (1955). At first, slight expansions appeared, which then drew apart, remaining connected for a time by an attenuating bridge of sarcoplasm, which eventually snapped. Transverse, longitudinal, and tangential clefts split the remaining sarcoblasts into short elements, which then separated as though by an oblique lateral shearing or slipping movement. Gradually, contractility was lost. The end result was a generalized fragmentation of the original muscle slips into widened segments of rhomboidal, fusiform, or clubbed shapes, which lacked the birefringence that characterizes the normal fiber, and in which the mitochondria no longer retained their longitudinally oriented position. In normal myoblasts which have assumed a strap-shape, the mitochondria are strictly aligned, as shown by Chambers and Fell with dark-field illumination. This disruptive process occupied from several hours to days; it was reversible, and recovery was possible when the drug was withdrawn, even after prolonged exposure. It is suggested by Godman that this series of effects are due to the action of colchicine on an oriented system of extended protein micelles, presumably actomyosin protofibrils, so as to disturb their relationships or internal configurations, or both.

3. *Fibrils and Banding*

Friedheim's conclusion (1931) that contractility is bound to cross-striation visible with polarized light has not been generally corroborated. Numerous short-term studies on living contracting fibers at the light microscope level have noted only a generalized birefringence paralleling the fiber axis. Though in long-term cultures elaborate

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FIG. 10. Branching fiber in 5-day culture. Magnification: × 550.

FIG. 11. Fiber in 14-day culture, apparently in process of fusion or of separation (arrows). Note banded fibrils continuing across gaps in sarcoplasm. Magnification: × 320.

FIG. 12. Sarcoblast with well-developed banded fibrils irregularly aligned. Fourteen-day culture. Magnification: × 1500.

Observations and photomicrographs for Figs. 7–10 inclusive are by Mr. Charles Capers and for Figs. 11 and 12 are by Dr. George G. Rose, from the Tissue Culture Laboratory of Dr C. M. Pomerat, University of Texas in Galveston .

The material is living chick skeletal muscle, visualized by phase contrast microscopy. From 13-day embryos.



PLATE II

FIG. 7. 8-Day culture, showing cross-banding in fibrillar arrangement, on each side of the nucleus. A juxta-nuclear area is occupied by clear vacuoles Magnification: × 1500.

FIG. 8. Branching or fusing sarcoblasts with irregular banding; fatty vacuoles 9-day culture. Magnification × 1600.

FIG. 9. Sarcoblast of 9-day culture with highly developed, comparatively regular banding. Arrows point to M bands. Magnification· × 1600.



PLATE III

FIG. 13. Differentiating mononuclear cell in living 30-day culture of adult human muscle. Fibrils are evident, with frayed appearance at anterior and posterior boundaries of the cell. Cross-banding is irregular. Magnification: × 450.

FIG. 14. Enlargement from same negative as in Fig. 13 (Magnification: × 1350). Arrow points to the same band in both figures

FIGS. 15 and 16. The same cell 24 hours later. Arrows point to banded fibril in formerly undifferentiated area of cell Magnifications: × 450 and × 1350.

FIGS 17 19. Human rhabdomyosarcoma, 3 days *in vitro*. Living, phase contrast. Magnification: × 100. Note extreme pleomorphism, with bizarre forms.

Observations and photomicrographs by Murray

patterns of cross-banding may eventually develop, their correlation with spontaneous contraction is imperfect. Sarcoblasts of all degree and even mononuclear myoblasts respond to mechanical stimuli by twitching; spontaneous contraction at all levels of differentiation tends to be sporadic and dependent on environmental factors rather than on structure as visible with the light microscope.

There is no doubt that in intact differentiating sarcoblasts there appear longitudinal structures which may reasonably be called fibrils; since they are unequivocably visible in the living state (Plate I, Figs. 2, 3) they cannot be regarded as fixation artifacts. Though they appear first in the body of the cell, usually near a nucleus, they eventually extend to the anterior and posterior borders, where they give the cell a frayed or fringed appearance. A sarcoblast packed with these structures is still quite plastic, as evidenced by changes in the surface configuration and in the position of the nucleus from day to day (Plate III, Figs. 13–16). After varying periods in culture, depending upon the maturity of the explanted tissue, cross-banding can be seen. This, too appears gradually, and involves at first only portions of the longitudinal fibrils. At first, it is of a primitive type in which only A and I bands can be discerned; later Z bands can be made out, and eventually the M–H region can be detected, the latter especially by phase microscopy. In these developmental stages, banded fibrils or bundles of fibrils appear to slip on one another. Sarcomeres do not extend regularly across a sarcoblast, as in the classic mature fiber that is used for paradigms. (Plate II, Figs. 7–12; Plate III, Figs. 13–16). This irregularity occurs in differentiating fibers, whether from explants of adult muscle (Pogogeff and Murray) or from embryonic material (Pomerat, Rose). It calls to mind the many observations of contracting *regions* of cells, which likewise indicate a lack of functional unity in development.

Morphological observations with the light microscope have lately and with justice given place to electron micrographs. However, in interpretation of ultrastructure and its correlation with development and function, as well as in scale orientation, tissue culture seems to retain a unique value because it offers living morphological material whose developmental history and behavior can be known and recorded at the cellular level before being prepared for electron microscopy. In the above accounts, an attempt has been made to provide a taking-off point for this type of exploration.

C. REGENERATION

The pathological anatomists of the late nineteenth century were aware of the considerable capacity of mammalian skeletal muscle to regenerate after necrotizing injury where the stromal pattern was retained as in typhoid fever. However, because of its abortive or slight regeneration after traumatic wounds and burns, the idea is still widespread that muscle has little inherent growth capacity once it has reached the differentiated state. Recent studies *in vivo* (Le Gros Clark, 1946) as well as the observations noted above should leave no doubt that mature mammalian skeletal muscle has a substantial potential for regenerative growth. It is probable that the actual extent of healing in any given case is conditioned more by extrinsic factors governing the stroma, scar formation, and reinnervation than by any intrinsic limitation of growth capacity.

When muscle injury occurs, the local inflammatory response coupled with the modulatory changes involved in the breakdown and reconstitution of these highly organized syncytial units produce a histological chaos which has been studied for a century without final resolution. The transformation of sarcoblasts to monocellular forms such as histiocytes, fibrocytes, and fat cells has often been proposed, also their disintegration into myoblasts which then redifferentiate in the embryonal manner. Budding and the reversion of the mature muscle to an undifferentiated embryonal blastema have likewise been described. Since studies on the behavior of muscle in tissue culture have the advantage of eliminating systemic factors which lead to confusion or indirectly affect regenerative growth, a number of investigators have approached regenerative problems by this means, among them Kikuchi (1931-1932), Mauer (1939), and Godman (1957).

Kikuchi explanted injured rabbit muscle at various intervals and described the appearance of muscle buds, muscle corpuscles, myophagic cells, and transitional forms between them. Mauer examined sections of explanted chick muscle and noted the formation from muscle fibers of spindle cells capable of fibrillogenesis, a process oddly called "regressive metaplasia." In the most comprehensive work of the three, Godman made comparative studies of healing lesions of rabbit muscle *in vivo* and of uninjured rat miscle *in vitro*, attempting to correllate the source and the destiny of the cell types appearing in the two situations. The events of regeneration and the cellular forms partici-

B. Modulation Range

In contrast to tissue *determination*—the progressive irreversible restriction of cellular potency with divergence of tissue type—which occurs during development, a less persistent and less far-reaching change in cellular form and function is recognized, and is known as *modulation*. This term encompasses the reversible changes, in response to external stimuli, that may be assumed by cells of a given tissue type, once this type is determined. In modulation capacity, skeletal muscle is particularly versatile, having a range of modulations that extend from the giant multinucleate forms (described by e.g., Hogue and de Rényi, 1939) to macrophage or histiocyte-like forms (studied intensively by Chèvremont, 1940, 1945, 1948). The series of changes undergone by embryonic muscle, from spindle-shaped myoblast to plasmodial structure and to cross-striated sarcoblast, have been recounted above; they are not necessarily permanent. Differentiation may regress as well as progress *in vitro*, and even cross-striation may be unstable and may appear and disappear more than once in the same cell.

When adult rat or human muscle is explanted (Pogogeff and Murray, 1946), a great variety of modulated forms may be observed (Plate I, Figs. 2–6; Plate III, Figs. 13–16): round, spindle, strap, rhomboid, trapezoid, ribbon, and even stellate or irregular shapes, which may vary greatly in size. After a lag period of 1–3 weeks, these wander or grow out from explants containing sarcoblasts in varying degrees of injury. This outgrowth therefore contains regressive forms mingled with differentiating forms. Cultures of adult rat muscle were maintained continuously by the authors for as long as a year and a half, during which time they displayed both differentiation and dedifferentiation, according to area and conditions. Contraction was present until the observations ended, but not continuously in the same groups of cells. The relation of these relatively gross changes in shape, visible organization, and apparent viscosity to muscle ultrastructure might be worth investigation. Could any of them be correlated with micellar organization or with G⇄F transformation? To what degree does the sarcolemma control external form, and how is its constitution involved in these changes?

scopically, a view that is in accord with Porter's (1951) electron micrographs of amphibian myoblasts in which such a "polymerization" is suggested.

D. Pathology

Morphological exuberance ensues when a form normally as highly organized as the skeletal muscle fiber escapes from restraining influences and yet remains viable and reproductive, as in neoplasia. Rhabdomyosarcomas explanted *in vitro* recapitulate as a group the modulation history of regenerating and developing muscle in culture, but rarely, if ever, reach the end point of cross-striation. Round forms, spindle forms, "fibroblasts," sarcoblastic ribbons which may or may not develop myofibrils, and giant multinucleate forms can be identified, though not all of these need appear in a single tumor. In addition to these conventional modulants, exceedingly bizarre forms are sometimes seen in very malignant tumors (Plate III, Figs. 17–19) which yet in their texture and outline generically resemble the cultured normal cells. An experienced observer of the modulation range that characterizes muscle *in vitro* can put this knowledge to use in tumor diagnosis, as Murray and Stout (1954, 1958) have done in the classification of tumors from a variety of tissue origins. For rhabdomyosarcoma, the differential diagnosis usually lies between this and liposarcoma or fibrosarcoma, neither of whose parent normal tissues runs a comparable gamut of modulation.

Under the spur of increased interest in muscular dystrophy as a disease entity, *in vitro* studies of dystrophic muscle, both mouse and human, have recently been undertaken. Geiger and Garvin (1957) report considerable differences between regeneration pattern and behavior of normal and dystrophic human muscle, but these findings have not been confirmed by U. R. Konigsberg (1958) as regards lag period, life span, and degree of differentiation attained by the two types of material. However, during the first two weeks of culture, Konigsberg found differences of unknown significance in the protein/DNA ratio. Since many factors influence the behavior of adult skeletal muscle *in vitro* and it is actually very variable on all the counts mentioned, the writer believes that ample quantitation should be applied and extreme caution observed in generalizing. For example, in a series of explants taken from various regions of the thigh and shank of the rat, Pogogeff and Murray (unpublished) found that explants

pating were found to be essentially similar *in vivo* and *in vitro*. Though the most significant mode of muscle regeneration was through terminal budding of fiber stumps, isolated fusiform elements with one or more nuclei might also survive and redifferentiate following nonlethal injury to fibers. The large numbers of round, macrophage-like cells which have repeatedly been described in muscle lesions and in tissue culture were regarded by Godman, however, as derived mainly from the fixed tissue histiocytes of the endomysium. Free macrophagic forms of whatever origin were distinguishable on the basis of cytological characteristics and their ability to store trypan blue intravitally. He found no evidence of a differentiation of this cell entity into histotypic muscle.

Godman notes that *in vivo* the macrophagic round cells are always concentrated close to the main necrotic masses where it might be expected that chemotactic agents released from degenerating muscle should be present in greatest abundance. Murray and Pogogeff (unpublished) have also noted, in a series of large and small muscle explants, that explants too large to be adequately nourished and oxygenated by diffusion in the given culture conditions evolve a relatively high ratio of macrophage types to sarcoblastic elements. This cell type can be distinguished from myoblast and other differentiating forms by cytological characteristics, but not as regards the ingestion of vital lithium carmine suspension, a process carried on also by myoblasts. According to Chèvremont and Chèvremont-Comhaire (1945), the histiocyte form and function may be assumed characteristically by many tissue types, and this transformation in muscle is governed by humoral factors identified as choline and related quaternary bases. They report further that it can be suppressed or eliminated by precipitation of choline or by administration of cholinesterase—an observation which should be rechecked in the conditions of Godman's experiments and our own.

As regards cytodifferentiation of regenerating adult muscle buds *in vivo*, Godman's observations differ from those of the Lewises' (1917) on embryonic chick material *in vitro*. As he points out, the budding sarcoblast in prefibril stage already possesses a high degree of organized structure. The myofibrils when they become visible usually appear to be in continuity with those of the parent fibers. He supposes that these fibrils originate from pre-existing submicroscopic protofibrils which gradually aggregate into cables of sufficient size to be visible micros-

fiber, which may be influenced not only by its developmental status but also by nutrition, oxygen tension, fatigue, and accumulation of wastes in the medium. Better defined factors such as pH, osmolarity, temperature, and electrolyte supply undoubtedly enter into this complex in shifting balance with advancing age and vicissitude in culture.

Investigating the relationship between electrical excitability and the appearance of spontaneous muscular activity, Szepsenwol (1947) subjected cultures of myogenic tissue in an age series to condenser discharge shocks. He found that the threshold declined as differentiation advanced, and was low when spontaneous activity began. Compared to skeletal muscle, cardiac muscle is precocious in developing spontaneous contraction; in this material he found that the threshold was low from the start of cultivation, and did not decline appreciably as differentiation proceeded. Such observations on excitability and spontaneous contraction call to mind the phenomena of fibrillation in denervated skeletal muscle (Tower, 1939) and pose the question whether nervous connections are regularly involved to some degree in inhibition of fiber contraction.

B. Drug Action

The key position held by the myoneural junction in chemical transmission and in the problems of "blocking" have made it of some interest to observe the effects of biologically active compounds on the behavior of living, uninjured muscle fibers with intact sarcolemma but entirely devoid of neural connections and end-plates. Experiments of this sort have been performed by Sacerdote de Lustig (1942, 1943), from which it was reported that physostigmine and low concentrations of acetylcholine activate the automatic contractility of chick skeletal and smooth muscle, while adrenaline, atropine, and high concentrations of acetylcholine paralyze it. Heart muscle activity is paralyzed by acetylcholine and stimulated by adrenaline; the acetylcholine paralysis is released by atropine. Several curarizing substances (curare, erythrine, snake venoms, strychnine, and veratrine) were also tested on skeletal and cardiac muscle. Both tissues were activated slightly by low concentrations (10^{-3}) and paralyzed at higher concentrations. Curare, erythrine, and cobra venom antagonize only the paralyzing action of acetylcholine, while strychnine and veratrine may reactivate cultures paralyzed by physostigmine-acetylcholine, erythrine, or curare.

from regions having a more abundant blood supply produced superior outgrowth as regards differentiation and contraction.

IV. PHYSIOLOGY

A. Spontaneous Rhythmic Contraction

In 1915, M. R. Lewis reported that rhythmical contraction of embryonic chick skeletal muscle occurs spontaneously in culture, entirely free from nervous influences. This observation has been repeatedly corroborated, and Pogogeff and Murray (1946) extended it to include adult mammalian material. Lewis found that replacing the nutrient balanced salt solution with physiological saline did not favor rhythmic contraction but produced a twitching which was usually followed by degeneration of the fiber. Dilution of the medium by 10% also resulted in loss of fiber form and degeneration. Chambers and Fell (1931–1932) noted that puncturing the cytoplasm with a microneedle caused a twitch, followed by a 10 sec. refractory period; occasionally, the contraction and thickening of the fiber were most pronounced about the pricked region, and the following relaxation was slow. De Rényi and Hogue (1934) also reported cases in which a mechanically rendered impulse appeared to travel only a short distance. Friedheim (1931) reported that the spontaneous contraction rhythm (chick) ranged from 40 to 90 per minute. In Pogogeff and Murray's rat preparations, the average rate was 138 per minute, with individual fibers in the same culture often contracting in different rhythms; it was observed that a portion of a fiber could contract, pulling the rest passively. Where the explant was found contracting as a whole, it also might be subdivided into areas with different rates.

The spontaneous activity of skeletal muscle is not continuous; a period of rhythmic contraction is followed by a rest interval. According to Szepsenwol (1946), the frequency of contraction and duration of rest periods for individual fibers from embryonic chick material are characteristic and stable for as long a time as 2 months *in vitro*. In the writer's experience, however, with late fetal, newborn, and adult rat material, the duration of rest periods is unpredictable, as is the rhythm of single fibers and groups of fibers from day to day. The response of resting or active fibers to chemical agents in the medium is similarly not uniform, especially in long-term cultures. One may suppose that *excitability can vary with the general physiological condition of the*

TABLE I

DRUG EFFECTS ON FETAL RAT SKELETAL MUSCLE IN VITRO.

Agent	Concentration (molar)	Concentration (mg. ml.)	Effect on contraction	Numerical rating
	3.10^{-3}	1.0	Not reversible	Toxic
Procaine HCl	3.10^{-4}	0.1	Inhibitory	— 7.1
	3.10^{-5}	0.01		— 1.6
	6.10^{-4}	0.1	Inhibitory	—11.2
Veratrine	6.10^{-5}	0.01		— 5.1
	—	0.1	Inhibitory	— 6.6
d-Tubocurarine	—	0.001	Inhibitory?	— 0.7
	—	0.0001	No effect	— 0.2
	—	0.00001	Stimulatory?	+ 1.2
				(Estimate)
	2.10^{-6}	0.001		— 8.
Ryanodine	2.10^{-7}	0.0001	Inhibitory	— 5.
	2.10^{-8}	0.00001		— 2.
ATP	5.10^{-3}		No effect	
	—	0.01	Inhibitory	— 3.8
Acetylcholine Cl⁻	—	0.001	No effect	+ 0.1
Caffeine	—	1.	Stimulatory?	+ 1.6
	—	0.1		+ 1.5
	—	0.001		+ 0.4
Mapharsen	—	0.0001	Stimulatory	+ 1.0
	—	0.00001		+ 3.2

no myoneural junction is necessary. Ryanodine, an alkaloid with some curare-like properties *in vivo*, appears to be membrane-active rather than being involved with actomyosin directly, as suggested by Edwards *et al.* (1948), who found that nervous control seemed not to be involved. Our experiments with ryanodine, undertaken to test this proposition, showed that at the concentration range noted in Table I, rhythmic contractions ceased within 15–45 min. after administration, time being inversely proportional to concentration; when the agent was withdrawn, recovery time was directly proportional to concentration. For this paralysis, nervous intervention is certainly not necessary; but ryanodine has no effect on nonconducting fiber preparations. Its total action, however, still has many unexplained aspects. Tissue culture thus appears to offer potentialities, especially if used in combination with other methods, for elucidating mechanisms of drug action in relation to contractility.

In all such preparations, there exists a substantial source of error, since spontaneous contractions may be sporadic in occurrence and not uniformly strong or rhythmic in different cultures from the same material. As an aid to the desired controlled quantitative observations, Pogogeff and Murray (unpublished) devised a simple arithmetical system of evaluation which is applied to cultures of late fetal or newborn rat muscle before and during an experiment. In this, three main categories (slight, good, and excellent) are based on the *number* of fibers contracting spontaneously. Each main category is further subdivided into five on the *strength* of contraction. This system allows subjective ratings (always applied by the same observer) from 0–16, which are given to the whole series of cultures at the beginning of an experiment. Rated cultures are then distributed as equably as possible between experimental and control groups, and during the exposure period they are periodically re-rated. Ratings for each group are totaled and averaged. If the rating for an experimental group increases significantly over that for the controls, the agent is said to be stimulating; if it decreases, the agent is said to be inhibiting. Since some confusion may arise from general toxicity of drugs apart from their specific pharmacological action, it should first be ascertained that agents are used at a concentration whose effects are reversible upon their withdrawal. Table I shows the action of eight biologically active compounds on this system *in vitro.*

From this, it may be concluded that in rat somatic muscle without innervation or end-plates, procaine, veratrine, tubocurarine, and ryanodine are inhibitory, with the possibility that at very low concentrations tubocurarine may be stimulatory, as noted by Sacerdote de Lustig; ATP and acetylcholine are without effect or inhibitory; and caffeine and mapharsen appear to activate contraction. The question still exists whether some or all of these compounds are able to penetrate the cell, or to affect membrane permeability or whether they exercise their effect from outside the membrane. Here a comparison with glycerol-frozen nonconducting fiber preparations from the rabbit (Korey, 1950) may assist interpretation. In these, cocaine and veratrine (classic membrane-active drugs) are without effect. So, also, are ryanodine and caffeine. Mapharsen is inhibitory and ATP, of course, stimulating. Here, as in earlier biochemical preparations, ATP reacts directly with the contractile substance of muscle.

It is clear that for the tested curarizing drugs to exert a blocking effect,

Recently, however, Crain (1956) has perfected a method of making intracellular action potential recordings of normal magnitude from single cells *in vitro*. This involves a modified Chambers-Pike microdissection assembly with oscilloscope hookup; KCl-filled glass electrodes of 0.5 μ diameter are inserted into neurons or muscle cells visualized with the high power compound microscope. With such a small electrode , the impaled cells may remain viable for a half hour or more. Typical resting and action potentials can be recorded from spontaneously contracting skeletal and cardiac cells, as shown in Crain's description and Figs. 20 and 21. Similar potentials have also

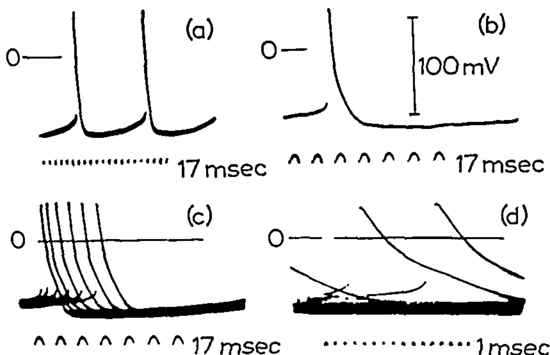


Fig. 21. Membrane resting and action potentials recorded intracellularly from a cultured, spontaneously contracting *skeletal* muscle fiber. Note the similarity in the mode of onset of the spikes to that of the cardiac fibers (Fig. 20), the large overshoot, and the "negative after-potential." The family of action potentials shown in C was obtained by nearly synchronizing the sweep frequency with that of the fiber discharges (analogous to Fig. 20, C_2). Age of culture: 9 days *in vitro*, from a newborn rat. Recordings were made by Dr. S. M. Crain at the College of Physicians and Surgeons, New York, 1955.

been obtained from trypsin-dissociated groups of cultured cardiac fibers (Fange *et al.*, 1956). Crain (1956) found in neurons that the response to electrical stimulation delivered 200–300 μ away from the cell is not exclusively of the all-or-none type, but that graded responses occur near the threshold. Friedheim, with the techniques available in

C. Bioelectric Phenomena

Many workers have made EKG records from tissue cultures, especially of contracting heart muscle. This extensive literature has been reviewed by Levi (*1934*) and discussed by Fell (*1951*). Usually, summation effects have been obtained with relatively large electrodes (30 μ), as by Szepsenwol (1916, 1947), who used both cardiac and skeletal muscle. In the latter material, a single, quick, bipolar wave was found where a group of fibers had the same frequency, but this was replaced with a slow mono- or bipolar wave when several different rhythms were present. In cardiac muscle cultures, Hogg and associates (1934–1935) obtained small extracellular pickups with electrode tips of the order of 2–5 μ, probably from single cells; they recorded positive monophasic spikes with an "overshoot" during the spike.

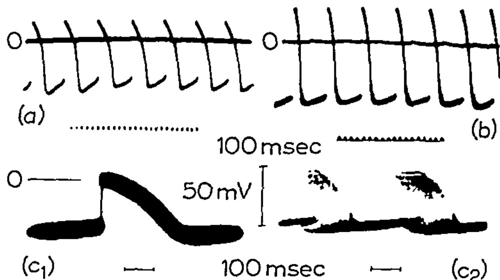


Fig. 20. Typical membrane resting and action potentials recorded with microelectrodes (tip diameter about 0.5 μ) inserted into the interior of cultured, spontaneously contracting *cardiac* muscle fibers. The reference level (O) was obtained by superimposing a sweep taken with the microelectrode tip just outside of the cell, immediately before (or after) the impalement. In this, as well as in Fig. 21 an up- [illegible] zation (the rising phase of the spike appearing as a discontinuity in the oscilloscope trace), the large overshoot, and the absence of any steady membrane potential between spikes (also *the small responses recorded in B just before effective penetration*), (C_1) series of superimposed action potentials obtained by synchronizing sweep frequency with that of the cardiac discharges, (C_2) two families of discrete curves obtained by slightly unbalancing this synchronization. Age of cultures: (A) 6, (B) 13, and (C) 4 days *in vitro*, from 9-day-old chick embryos. Recordings were made by Dr. S. M. Crain at the College of Physicians and Surgeons, New York.

medium. The significance of this observation can only be surmised; it may be related to an irregular series of later observations made as the use of simplified media has developed. Fischer's V-614 amino acid mixture (1948) plus a little balanced saline extract of chick embryos is especially favorable for embryonic skeletal muscle (K. R. Porter, personal communication). In White's chemically defined medium (1949), consisting chiefly of salts, amino acids, and vitamins, muscle is conspicuous among mixed embryonic tissues for its survival and continued function. Waymouth reports in a personal communication that a variant of her defined medium 192/2 with the addition of peptone (Waymouth, 1956) especially favors contraction in skeletal muscle; this applies even to myoblasts and multinucleate round cells, which contract radially. According to Godman (1957), muscle fibers grown in synthetic medium 858 (Healy *et al.*, 1955) with the addition of saline embryo extract differentiate precociously. If there is a unifying thread here, it is the presence of balanced saline solution and amino acids in considerable amounts, and the absence of whole serum as a constituent of the medium; these recurrent independent factual observations seem worthy of note.

Recently, at the Strangeways Laboratory, a series of studies has been undertaken on growth and metabolism. The tissues are grown in protein-free media of known chemical composition (Parker's 858), and the utilization of amino acids is determined quantitatively by column chromatography. When suspensions of embryonic chick muscle cells are cultivated in a medium containing amino acids in equimolar concentrations, leucine, isoleucine, and aspartic acid are found to be used in large amounts. The relative amounts used agree well with the proportions in which these compounds are present in muscle proteins (Lucy and Rinaldini, 1959). Studies on the synthesis of nonessential amino acids from C^{14}-labeled glucose in natural media indicate that alanine, aspartic acid, glutamic acid, serine, glycine, and proline can be synthesized by skeletal muscle, as well as by limb bones *in vitro* (Rinaldini and Webb, 1956).

The accumulation of glycogen in differentiating skeletal muscle (van Weel, 1948) was mentioned earlier in another connection. Sacerdote de Lustig and Mancini (1946) have pursued this subject somewhat more extensively, finding similarly that embryonic chick muscle is capable of synthesizing glycogen *in vitro*, and that the speed of glycogen production is related directly to the age of the embryo at

1931, reports similarly that the contraction magnitude of the isolated skeletal muscle fiber, when stimulated electrically, is a function of the magnitude of the current. In this situation, the all-or-none rule does not apply.

The technique of recording action potentials from impaled cultured cells, as handled by Crain (1956) and Fange *et al.* (1956), could contribute important electrophysiological data if combined with the administration of biologically active compounds *in vitro*. Also, in view of the large potentials recorded with normal cells, it seems probable that the method could be utilized to investigate intrinsic differences in functional capacity in developmental or in pathological states.

V. NUTRITION AND METABOLISM

Investigation of nutrient requirements and metabolic exchanges of the special tissue types is just in its beginnings. Though from the early days of tissue culture attempts have been made to replace the "natural" media of body fluids, in which explanted animal tissues flourish, with media of known chemical constitution, only in the last dozen years has the goal been closely approached. Recent reviews of the general field of nutrition *in vitro*, to which the reader might refer, are those of Waymouth (1954), Biggers *et al.* (1957), and Morgan (1958).

Much of the early nutritional work concerned itself with "growth factors", and used as cultured material sarcoma cells or heart "fibroblasts" whose histogenesis was obscure and which for many generations had been maintained in a state of rapid multiplication that precluded the assumption of a differentiated form or function. Nevertheless, Parker (1933) noted quantitative differences in nutrition and growth rate between strains of "fibroblasts" from different sources, skeletal muscle among them.

The earliest attempts to maintain animal cells in simplified, relatively defined media as contrasted to cell extracts and body fluids were made by the Lewises, who accomplished an enormously productive series of morphological and physiological studies on a great variety of tissues. Cultures were carried for periods usually less than a week in a mixture of balanced salt solution (Locke), glucose, and bouillon or peptone. In their studies of skeletal muscle (Lewis and Lewis, 1917), they noted that spontaneous contraction was much more marked than it appeared to be in the cultures of contemporaries who employed a plasma

Chèvremont, M., and Chèvremont-Comhaire, S. (1945). *Acta Anat.* **1**, 95.
Crain, S. M. (1956). *J. Comp. Neurol.* **104**, 285.
Crain, S. M. (1955). *In* "Electrical Activity in Tissue Cultures of Chick Embryo Spinal Ganglia," Univ. Microfilms, Ann Arbor, Mich. 10, 785, 121 pp.
de Rényi, G. S., and Hogue, M. J. (1934). *Arch. exptl. Zellforsch. Gewebezücht.* **16**, 167.
Edwards, G. A., Weiant, E. A., Slocombe, A. G., and Roeder, K. D. (1948). *Science* **108**, 330.
Fange, R., Persson, H., and Thesleff, S. (1956). *Acta Physiol. Scand.* **38**, 173.
Fell, H. B. (1951). *In* "Cytology and Cell Physiology" (G. Bourne, ed.), Chapt. 10. Oxford Univ. Press, London and New York.
Fischer, A. (1948). *Biochem. J.* **43**, 491.
Frédéric, J. (1954). *Compt. rend. soc. biol.* **148**, 621.
Friedheim, E. A. H. (1931). *Arch. exptl. Zellforsch. Gewebezucht.* **11**, 303.
Geiger, R. S., and Garvin, J. S. (1957). *J. Neuropathol. Exptl. Neurol.* **16**, 532.
Godman, G. C. (1955). *Exptl. Cell Research* **8**, 488.
Godman, G. C. (1957). *J. Morphol.* **100**, 27.
Godman, G. C., and Murray, M. R. (1953). *Proc. Soc. Exptl. Biol. Med.* **84**, 668.
Healy, G. M., Fisher, D. C., and Parker, R. C. (1955). *Proc. Soc. Exptl. Biol. Med.* **89**, 71.
Hogg, B. M., Goss, C. M., and Cole, K. S. (1934-1935). *Proc. Soc. Exptl. Biol. Med.* **32**, 304.
Hogue, M. J., and de Rényi, G. S. (1939). *Arch. exptl. Zellforsch. Gewebezücht.* **23**, 122.
Holtfreter, J. (1947). *J. Morphol.* **80**, 57.
Holtzer, H., Abbott, J., and Cavanaugh, M. W. (1959). *Expl. Cell Research* **16**, 595.
Kikuchi, S. (1931-1932). *Mitt. allgem. Pathol. u. pathol. Anat. Sendai* **7**, 179.
Konigsberg, I. R. (1958). *Tissue Culture Assoc. Symposium Phila.* 8.
Konigsberg, U. R. (1958). *Tissue Culture Assoc. Symposium Phila.* p. 8.
Korey, S. (1950). *Biochim. et Biophys. Acta* **4**, 58.
Lake, N. C. (1915). *J. Physiol. (London)* **50**, 364.
Le Gros Clark, W. E. (1946). *J. Anat.* **80**, 24.
Levi, G. (1934). *Z. ges. Anat. Abt. III. Ergeb. Anat. u. Entwicklungsgeschichte* **31**, 125.
Levi, G. (1954). "Trattato di Istologia," Vol. 2, p. 627. Unione Tipografico Editrice Torinese.
Levi, G., and Chèvremont, M. (1941). *Arch. biol. Paris* **52**, 523.
Lewis, M. R. (1915). *Am. J. Physiol.* **38**, 153.
Lewis, M. R. (1920). *Carnegie Inst. Contribs. Embryol. No.* 35, **9**, 191.
Lewis, W. H. (1923). *Anat. Record* **26**, 15.
Lewis, W. H., and Lewis, M. R. (1917). *Am. J. Anat.* **22**, 169.
Lucy, J. A., and Rinaldini, L. M (1959). *Exptl. Cell Research in press.*
Mauer, G. (1939). *Arch. exptl. Zellforsch. Gewebezucht.* **23**, 125.
Morgan, J. F. (1958). *Bacteriol. Revs.* **22**, 20.
Moscona, A. (1956). *Proc. Soc. Exptl. Biol. Med.* **92**, 410.
Moscona, A. (1958). p 49 *in* "Cytodifferentiation" (D. Rudnick, ed.) [International Symposium on Cytodifferentiation, Brown University, 1956]. University of Chicago Press.
Mutchmore, W. B. (1951). *J. Exptl. Zool.* **118**, 137.
Murray, M. R., and Kopech, G. (1953). "A Bibliography of the Research in Tissue Culture 1884 to 1950," Vols. 1, 2 Academic Press, New York.
Murray, M. R., and Stout, A. P (1954). *Texas Repts. Biol. and Med.* **12**, 898.
Murray, M. R , and Stout, A. P. (1958) *In* "Treatment of Cancer and Allied Diseases" (G. T. Pack and I. M. Ariel, eds.) Chapt. 8. Hoeber, New York.
Parker, R C. (1933). *J. Exptl. Med.* **58**, 401.
Parker, R. C. (1950). "Methods of Tissue Culture." Hoeber, New York.

explantation. They found no relation between growth rate and glycogen production, nor could they observe a close correlation between content of glycogen and spontaneous contraction.

In the foregoing discussion of muscle tissue in culture, no general exposition of tissue culture method has been included. It is assumed that readers are familiar with the underlying principles; for more comprehensive treatment of technical matters, reference can be made to compendia such as those of Parker (1950), Pomerat (1951) and Paul (1959). The physical resources of the tissue culture method have been expanding with great rapidity in the period since World War II, so much so that new editions of most standard works, and several entirely new productions, are now in preparation. Development has followed two main lines: (1) intensive cell propagation producing massive cultures for use in the study of growth, nutrition, metabolism, genetic alteration, and malignancy; and (2) spatial restriction of cell growth *in vitro* directed toward fostering morphogenesis and function.

In this chapter devoted mainly to an account of observations on living contractile tissue, skeletal muscle has been considered selectively, as representing the greatest specialization of the contractile function, which is possessed in some degree by all protoplasm. Observations on cardiac muscle in culture are generically similar to those on striated; smooth muscle has not been studied so intensively. Restriction of space has made it necessary to omit more than occasional reference here to these latter types. Interested readers might profitably consult the fourth edition of Levi's Treatise on Histology (1954), in which the author devotes some seventy pages to an exposition of the histophysiology of the three divisions of muscle, illuminated by his fifty years of research in the general field of tissue culture. The Bibliography of the Research Tissue Culture (Murray and Kopech, 1953) is recommended for further reference coverage.

References

Avery, G., Chow, M., and Holtzer, H. (1956). *J. Exptl. Zool.* **132**, 409.
Biggers, J. D., Rinaldini, L M., and Webb, M. (1957). *Symposia Soc. Exptl. Biol.* **11**, 264.
Burrows, M. T. (1912). *Munch. med. Wochschr.* **59** 1473.
Chambers, R., and Fell, H. B. (1931-1932) *Proc. Roy. Soc.* **B 109**, 380.
Chèvremont, M. (1940). *Arch. biol. Paris* **51**, 313
Chèvremont, M. (1945). *J. Morphol* **76**, 139
Chèvremont, M (1948). *Biol. Revs. Cambridge Phil Soc.* **23**, 267.

CHAPTER VI

The Structure of Striated Muscle As Seen by the Electron Microscope[1]

H. Stanley Bennett

I. INTRODUCTION

Striated muscle has been extensively studied with the electron microscope. Many of these areas of study fall naturally under the headings of some of the chapters contributed to this book by other authors. Thus, in Volume I, Chapter IX, Hanson and Lowy discuss the structure of invertebrate striated muscle as seen with the electron microscope. In Volume I, Chapter VII, Huxley and Hanson present evidence derived from electron microscopy which bears on the molecular structure of vertebrate and invertebrate striated muscle and their changes in contraction. Volume I, Chapter XII consists of a description by Sjöstrand and Andersson-Cedergren of the intercalated discs of heart muscle. In these cases, much of the important evidence is derived from electron microscopy.

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Paul, J. (1959). "Cell and Tissue Culture." Livingstone, Edinburgh and London; Williams and Wilkins, Baltimore.
Pogogeff, I. A., and Murray, M. R. (1946). *Anat. Record* **95**, 321.
Pogogeff, I. A., and Murray, M. R. (1949). *Anat Record* **103**, 495.
Pogogeff, I. A., and Murray, M. R. (1951). *Anat Record* **109**, 338.
Pomerat, C. M. (1951). *Methods in Med. Research* **4**, 198.
Porter, K. R. (1954). *In* "Dynamics of Growth Processes" (E. J. Boell, ed.). Princeton Univ. Press, Princeton, New Jersey.
Rinaldini, L. M. (1957). *Intern. Tissue Culture Meeting Glasgow p.* **32.**
Rinaldini, L. M., and Webb, M. (1956). *Strangeways Research Lab. Repl. p.* **9.**
Sacerdote de Lustig, E. (1942). *Rev. soc. arg. biol.* **18**, 524.
Sacerdote de Lustig, E. (1943). *Rev. soc. arg. biol.* **19**, 159.
Sacerdote de Lustig, E., and Mancini, R. E. (1946). *Rev. soc. arg. biol.* **22**, 267.
Szepsenwol, J. (1946). *Anat. Record* **95**, 125.
Szepsenwol, J. (1947). *Anat Record* **98**, 67.
Tower, S. (1939). *Physiol. Revs.* **19**, 1.
Waymouth, C. (1954). *Intern. Rev. Cytol.* **3.**
Waymouth, C. (1956). *J. Nat. Cancer Inst.* **17**, 315.
van Weel, P. B. (1948). *J. Anat.* **82**, 49.
White, P. R. (1949). *J. Cellular Comp. Physiol.* **34**, 221.
Wilde, C. E. (1958). *Tissue Culture Assoc. Symposium on Aspects of Morphogenesis Philadelphia; Anat. Rec.* **132**, 517.

component and to coin a new term for the cell membrane of muscle (Péterfi, 1913; Gutmann and Young, 1944). However, in this chapter, the concepts and terminology of Schwann and of Bowman are used and are found to provide a suitable basis for the interpretation of electron micrographs of muscle. On these premises, the term sarcolemma is used for the membrane component of muscle corresponding to the cell or plasma membrane, to which selective semipermeable characteristics can most reasonably be attributed, and across which appears the resting potential of the muscle fiber (Katz, 1948, 1956). A somewhat more extended discussion of the significance of the term "sarcolemma" can be found in a previous review by Bennett (1958b).

B. Fine Structure of Sarcolemma

The sarcolemma was first studied with the electron microscope by Jones and Barer (1948) and by Reed and Rudall (1948). These two early studies appeared before the development of satisfactory sectioning methods, and depended on surface views of the sarcolemma. The sarcolemma was identified as a thin membrane underlying the collagenous connective tissue investment of the fiber. It was this collagenous investment which Péterfi (1913) confused with the sarcolemma itself. The observations of Jones and Barer and of Reed and Rudall confirmed Bowman and Schwann in that the sarcolemmatic membrane was structureless at levels of resolution accessible to the light microscope. But with the electron microscope, some inhomogeneities were observed. These were described as raised "spots" (Jones and Barer) or as "corpuscles" (Reed and Rudall) which were deemed to lie on an underlying membrane. These densities or elevations were said to be between 0.04 μ to 0.1 μ in diameter, and were scattered rather irregularly over the membrane itself. These "spots" have not figured in more recent descriptions of the sarcolemma based on sectioning. It is quite possible, however, that they may correspond to small vesicles and caveolae which have been seen in association with the sarcolemma (Porter and Palade, 1957) and which are illustrated in Figs. 35, 37, 38, 40, 42, and 45 (see p. 170, 172). These are taken from the work of Dr. David Robertson, Fawcett and Selby (1958), and of Porter and Palade (1957).

The sarcolemma has been described in sectioned striated muscle by Bennett and Porter (1953), Bennett (1955, 1958b), Ruska (1954), Robertson (1956a, 1957b), Porter and Palade (1957), and Fawcett and

It then falls within the compass of this chapter to discuss those phases of striated muscle structure which have been studied with the electron microscope, and which are not covered in other sections of this book. The main topics to be discussed here include the sarcolemma, the sarcoplasm, including the sarcoplasmatic reticulum and the sarcosomes or mitochondria, and the nuclei. Very brief attention will be dispensed on the important topic of myofibrils and myofilaments, which are described in detail in Volume I, Chapter VII; on the tracheoles of insect muscle, which fall in the province of Volume I, Chapter IX, and on the intercalated discs of cardiac muscle, which comprise the main topic of Volume I, Chapter XII. Vertebrate cardiac muscle is a type of striated muscle, and will be a subject of discussion of this chapter, except for those aspects of vertebrate cardiac muscle which fall within the domains of Volume I, Chapters VII and XII.

II. SARCOLEMMA

A. General Features

In defining the sarcolemma as it appears in electron micrographs, we draw on the findings of Schwann (1839) and of Bowman (1840). These authors described a thin, structureless membrane investing the striped muscle fiber. Both of them recognized the membrane as *distinct from the fibrous connective tissue component of the muscle.* Schwann spoke of it as corresponding to the cell membrane of the muscle fiber. Bowman, who named it the sarcolemma, wrote his paper before Schwann's book on the cell theory appeared, and was unaware of the concept of the cell at the time his paper went to press. He demonstrated the semipermeable character of the sarcolemma by observing a swelling of the muscle fiber and a lifting of the sarcolemma from the myofibrils when the fresh fiber was placed in water. When subsequently placed in strong sugar solution, the water previously imbibed was removed and the original appearance of the fiber was restored. In a footnote added in proof, Bowman states that he learned after sending his paper to press that Schwann had described the membrane which Bowman called the sarcolemma, and that Schwann had deemed it to be the cell membrane of muscle.

This brief review of work over a century old is pertinent here, as the nature of the sarcolemma has not been understood by all authors, and some have attempted to apply the term to a connective tissue

similarity of structure of many biological cell membranes, should be taken in context with the work of Fernández-Morán and Fincan (1957), Robertson (1955), Geren and Schmitt (1954, 1955), Schmitt and Bear (1939), Bear and Schmitt (1936), Bear *et al.* (1941), and others. These studies demonstrate that the myelin sheath consists of many layers of Schwann cell plasma membrane, and that this multiple stack of cell membranes is comprised of protein sheets alternating with polar lipid groups arranged radially with their hydrocarbon chains arrayed parallel to each other and normal to the planes of the protein sheets. The similarity between the sarcolemma and the Schwann cell plasma membrane, discerned by Robertson, permits one to surmise a similar model for the sarcolemma. In Fig. 39 one can see one of Robertson's pictures of the Schwann cell membrane and myelin sheath. This can be compared with the sarcolemmatic membranes seen in Figs. 34-36 and 40.

C. Configuration of Sarcolemma

A second important characteristic of the sarcolemma is that it is not a perfectly smooth cylindroidal membrane, but is marked here and there by tunnel- or cavelike invaginations or open vesicles called "*caveolae intracellulares*" (Yamada, 1955). They can be seen in Figs. 35, 37, 40, and 45 (C). Entirely analogous caveolae in a Schwann cell are displayed in Fig. 39. Caveolae have been discerned in mammalian smooth muscle sarcolemma by Mark (1956) and by Palade (1956), in cardiac muscle by Moore and Ruska (1957) and by Fawcett and Selby (1958), and in striated skeletal muscle by Porter and Palade (1957). Associated with these caveolae are vesicles which may lie close to the sarcolemma or even touch it without actually opening to the outside. These vesicles and caveolae are usually oval or ellipsoidal, but may have rather complicated ramifying and branching excursions (Palade, 1956, Plate 38, Fig. 8). They are usually about 600 A. in diameter and may be nearly spherical or elongated with a length greatly exceeding the diameter.

Most of the authors who have observed these small vesicles and caveolae associated with the sarcolemma have speculated on their function. The predominant view is that they may have a role in transport or transfer of substances into and out of the muscle cell. The general theory of this mechanism has been outlined by Bennett (1956a), and many features of it are discussed by Palade (1956). In essence, the

Selby (1958). The gross features of the sarcolemma as currently revealed in electron micrographs are shown in Figs. 37, 38, and 42–45, which have been supplied most generously by Drs. Porter and Palade and by Drs. Fawcett and Selby. At this resolution, the sarcolemma (S) appears as a thin dark line not over 100 A. in thickness. In more recent micrographs (see Figs. 34, 35, 36, and 40), Robertson (1957b, 1958a) has resolved this membrane as displaying two peaks of density about 50–60 A. apart, between which lies a trough of lesser density. Robertson (1957b, 1958a, b) has found that this trilaminate structure is characteristic of animal cell membranes in general. He speaks of it as the "unit membrane" structure, and has found it to be a feature of the plasma membranes of many types of cells, including Schwann cells, nerve cells, and muscle cells. Moreover, this "unit structure" is likewise characteristic of internal membranes in cells, and has been noted by Robertson in the membranes of mitochondria, endoplasmic reticulum, Golgi bodies, and nuclear envelope.

Similar pictures of twin densities within mitochondrial membranes have been obtained by Freeman (1956). Zetterqvist (1956) has demonstrated double parallel density peaks in the plasma membrane of the absorbing cells of the intestinal mucosa of the mouse, and Low (1956a, b) has reported this type of membrane structure in the plasma membranes and endoplasmic reticulum of the intestinal mucosa cells of the rat.

From these developments, and from Figs. 34, 35, 36, and 40, we can conclude that, surrounding the striated and smooth muscle fiber, there is a membrane which resembles closely in structure the plasma membrane and the internal membranes of other cells. It is altogether reasonable to regard this membrane as the plasma membrane of the muscle fiber recognized by Schwann and to follow Bowman in calling this membrane the sarcolemma.

In this light, the most revealing pictures of the sarcolemma itself are probably those of Robertson, several of which have been supplied most generously by Dr. Robertson and are reproduced in this chapter, with his kind permission. It can be seen from Figs. 34–36 that the overall thickness of the sarcolemma is somewhere between 50 and 100 A. In most places, it seems to be about 75 A. thick. Each of the two peaks in density is about 25 A. wide, and the intervening less dense portion measures about 20–25 A. in width.

The important unifying contribution of Robertson, relating to the

festations of active transport mechanisms. However, in other cells, caveolae and vesicles have been shown to have a role in active transport processes. Odor (1956) has observed transport of particles across mesothelial cells in vesicles and caveolae, and Hampton (1958) has demonstrated a similar transport mechanism to operate in hepatic parenchymal cells, carrying colloidal particles from plasma to bile canaliculi. Wissig (1958) and Alksne (1959) have noted that colloidal particles can pass across capillary endothelial cells in vesicles. Chapman-Andresen and Holter (1955) and Chapman-Andresen and Prescott (1956) reported that uptake of glucose and proteins by amoeba was accomplished by pinocytosis, using a vesiculation mechanism. These observations on cells other than muscle call attention to the possibility that vesicles and caveolae associated with the sarcolemma may have a role in ionic movements and in passage of glucose and other metabolites across the plasma membrane of the muscle cell. Experimental evidence of such a role for sarcolemmatic vesicles and caveolae is awaited. But in support of the concept, one may cite the work of Wotton and Mosti (1955), who noted droplets of stained fat passing through the sarcolemma of cardiac muscle cells of the cat, and the observations of Coons (1953), who reported pneumococcus polysaccharide to be present in cardiac muscle cells after intravascular administration. These experiments indicate that large molecules and fat droplets large enough to be seen with the light microscope can traverse the sarcolemma, indicating that a vesiculation mechanism may be involved. Thus it is reasonable to suggest that sarcolemmatic vesicles and caveolae may serve to transfer smaller molecules across the plasma membrane of muscle cells, just as similar structures function in amoeba.

D. Associated Extracellular Features

A third feature of the sarcolemma is an associated extracellular component, discernable in Figs. 34, 35, 37–40, and 42–44 as a cloud of material perhaps 500 A. thick, of moderate density, diminishing gradually in density as it extends further away from the sarcolemma into the intracellular space. This extracellular component is designated by the letter B in the figures accompanying this chapter, and is considered to be the representative in muscle of a structure known as "basement membrane," which invests certain surfaces of endothelial and epithelial cells.

concept holds that substances such as ions, sugars, proteins, or larger particles might gain access to the interior of cells or be discharged from cells without passing through pores in the plasma membrane and without risk of violating even momentarily the integrity of the osmotic properties of the cell. The substances would enter the cell in vesicles lined with membrane similar to or identical in composition to the plasma membrane. In the case of the uptake of materials, the concept holds that substances might first be bound to the outer surface of the plasma membrane of the cell. Specificity of uptake would depend on specificity of binding sites on the cell surface. After such binding, the membrane would infold to form a caveola; the orifice of this cave would seal, forming a vesicle, and the vesicle would become detached from the plasma membrane and take a place in the cytoplasm of the cell. Once in the cytoplasm, the vesicle membrane might be modified, disassembled, or destroyed, so that the substances become free in the cytoplasmic matrix. Alternatively, the contents of the vesicle might escape to the cytoplasm by diffusion or turnover of molecular components of the membrane. Or, the vesicle might be transported to another part of the cell, there perhaps to fuse with one of the interior membranes of the cell, such as those of some internal vesicle or vacuole, or of the endoplasmic or sarcoplasmatic reticulum or nuclear envelope, or of the plasma membrane in another part of the exterior surface of the cell. Upon such fusion, the contents of the vesicle would be liberated to the cavity or vesicle enclosed by the internal membrane or to the exterior of the cell, according to the location of the membrane with which the fusion took place. In the event that substances are to be discharged from the cell by this mechanism, the theory envisions that they first be enclosed in a vesicle within the cytoplasm of the cell. The enclosed material in the vesicle is then moved to the plasma membrane of the cell where the vesicle and plasma membranes fuse. Thereupon the membrane of the vesicle opens up in the form of a caveola and the contents are liberated to the exterior. An interesting feature of this mechanism is that particles, molecules and ions of widely varying size can move from one side of the plasma membrane to the other without the existence of any pores, or without violating the permeability characteristics of the membrane, or without actually going through the membrane.

No one has demonstrated that the caveolae and vesicles associated with the sarcolemma of muscle are actually morphological mani-

festations of active transport mechanisms. However, in other cells, caveolae and vesicles have been shown to have a role in active transport processes. Odor (1956) has observed transport of particles across mesothelial cells in vesicles and caveolae, and Hampton (1958) has demonstrated a similar transport mechanism to operate in hepatic parenchymal cells, carrying colloidal particles from plasma to bile canaliculi. Wissig (1958) and Alksne (1959) have noted that colloidal particles can pass across capillary endothelial cells in vesicles. Chapman-Andresen and Holter (1955) and Chapman-Andresen and Prescott (1956) reported that uptake of glucose and proteins by amoeba was accomplished by pinocytosis, using a vesiculation mechanism. These observations on cells other than muscle call attention to the possibility that vesicles and caveolae associated with the sarcolemma may have a role in ionic movements and in passage of glucose and other metabolites across the plasma membrane of the muscle cell. Experimental evidence of such a role for sarcolemmatic vesicles and caveolae is awaited. But in support of the concept, one may cite the work of Wotton and Mosti (1955), who noted droplets of stained fat passing through the sarcolemma of cardiac muscle cells of the cat, and the observations of Coons (1953), who reported pneumococcus polysaccharide to be present in cardiac muscle cells after intravascular administration. These experiments indicate that large molecules and fat droplets large enough to be seen with the light microscope can traverse the sarcolemma, indicating that a vesiculation mechanism may be involved. Thus it is reasonable to suggest that sarcolemmatic vesicles and caveolae may serve to transfer smaller molecules across the plasma membrane of muscle cells, just as similar structures function in amoeba.

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Indeed, it is timely to relate this structure in a broader context. Most cells, if not all cells, appear to adorn themselves with a polysaccharide-rich coating outside the plasma membrane. This coating may be very thin, as in the case of mammalian erythrocytes, where it represents surface coatings responsible for the A-B-O agglutination reactions demonstrable by immunochemical methods (Kabat, 1956). At another extreme, very thick coatings are represented in the cellulose-containing cell walls of higher plants, in the cell walls of fungi and bacteria, in the chitinous covering of arthropods, and in the zona pellucida of the mammalian ovum. The polysaccharides represented in such coatings are variable, including cellulose, polyglucosamine, polyglucans, hyaluronic acid, chondroitin sulfate, and many others. The polysaccharides may be mixed or conjugated with lipid, protein, polyphenols, lignin, or other polymers. The presence of such a thick or thin polysaccharide-containing coating just outside the plasma membrane of plant and animal cells is sufficiently common to justify recognition of the coating as a general structural feature of cells.

Turning now to striated muscle cells, the coating is represented by the parasarcolemmatic densities mentioned above. This density often shows a rounded summit perhaps 200-250 A. outside the sarcolemma itself, and a trough about 100 A. wide just outside the sarcolemma. It appears to correspond to the structure mentioned by Schiebler (1953) as responsible for the positive periodate-Schiff reaction associated with the sarcolemma. Outside this layer, one can often distinguish some collagen filaments (Fig. 43, F).

This coating, which is here called the basement membrane, may have a role in permeability and exchange phenomena of the muscle cell. Its composition, as represented in muscle cells, is not well understood, but it is presumed to contain carbohydrate and protein. It appears to be nearly structureless, even at high resolution, though at times a fine fibrillar appearance can be noted. It may be capable of binding ions or glucose and of concentrating such molecules close to the sarcolemma. It may be able to influence or regulate the molecules which are close to the outer surface of the sarcolemma. Some of the basement membrane material appears to become enclosed in caveolae and vesicles formed by invagination of the sarcolemma. Thus it appears to be a flexible and malleable structural component.

E. Experimentally Induced Changes in Sarcolemma

The sarcolemma appears to undergo appreciable changes in configuration under the influence of certain pharmacological agents. Thus Lindner (1957) has reported that, under the influence of strophanthin, a vigorous membrane activity with extensive vesiculation becomes evident. This important study indicates that the electron microscope may become a useful instrument in pharmacological research and that fine structure changes induced by physiological and pharmacological influences may improve our knowledge of cell physiology.

F. Regional Specializations of Sarcolemma

All regions of the sarcolemma are not identical in structure and physiological behavior. Thus, in many striated muscle cells, most regions of the sarcolemma are excitable electrically, but the sarcolemma underlying the motor end-plate is not excited by electrical pulses. Conversely, the sarcolemma of the myoneural junction can be excited by acetylcholine, whereas most of the area of sarcolemma of a striated muscle cell is insensitive to acetylcholine (Katz, 1956). These physiological differences indicate important distinctions in molecular structure, details of which, however, are concealed from our knowledge at the present time. But even with the relatively crude electron microscope, some regional structural differentiation of the sarcolemma can be detected. Here mention will be made of two such regions, though neither of them will be described minutely in this chapter.

1. *The Sarcolemma of the Motor End-Plate*

The structure of the motor end-plate is described in Volume I, Chapter X of this volume. The most definitive electron microscope study of the motor end-plate so far published is that of Robertson (1956b). A second important electron microscope study of this region has been published by Edwards and associates (1958). This second paper deals with motor end-plates in the leg muscles of the wasp. In this form, the sarcolemma is not conspicuously specialized morphologically in the region underlying the nerve ending, insofar as it can be studied with the electron microscope. Here the sarcolemma may be slightly thicker and more dense than elsewhere, but otherwise appears much as it does over other portions of the muscle cell.

In contrast, the sarcolemma underlying the motor nerve endings in

vertebrate muscle is elaborately specialized structurally. Robertson's electron micrographs show that the sarcolemma and basement membrane are here thicker and more dense than elsewhere and are folded in a series of troughs or grooves indenting the sarcoplasm of the fiber. The basement membrane material intervenes everywhere between sarcolemma and synaptic neural endings and between the membrane folds forming the grooves mentioned above. Robertson (1956b) has prepared a diagram showing these relationships, which are also discussed more fully in Volume I, Chapter X.

2. *The Sarcolemma of the Myotendinal Junction*

The myotendinal junction is described more fully in Volume I, Chapter II. No definitive electron microscope studies of myotendinal junctions have been published. Our information about the fine structure of this interesting region is therefore very meager. From unpublished micrographs, it appears that the sarcolemma of the myotendinal junction is specially modified, and in it are inserted tapering bundles of keratin-like filaments which are continuous with the contractile myofilaments at the first Z band. The sarcolemma is thicker and more dense at the regions of insertion than elsewhere. These insertions resemble the attachment of the keratin filaments of human epidermal cells to the cell membrane in the region of the attachment disc or node of Bizzozero, as described by Odland (1958). Opposite the filament attachments, the extracellular basement membrane is likewise thickened, and into these thickenings insert collagenous fibers of the tendon.

A more specialized type of myotendinal junction is represented in the intercalated discs of heart muscle, which are described in Volume I, Chapter XII. In these cases, a muscle fiber on one side of an intercalated disc functions as a tendon for its fellow on the other side. The intracellular filaments insert in the sarcolemma much as in ordinary myotendinal junctions, but the collagenous extracellular tendon is missing. In its place is another cardiac muscle fiber with a similarly specialized sarcolemma.

III. THE SARCOPLASM

A. General Features

If one defines the sarcoplasm of a muscle fiber as consisting of the contents of the sarcolemma exclusive of the fibrillar contractile

material and the nuclei, it is convenient to consider the sarcoplasm as having five main components. These are the sarcoplasmatic matrix, the mitochondria or sarcosomes, the sarcoplasmatic reticulum or tubular system, the sarcoplasmatic lipid bodies, and the Golgi apparatus. In insect muscle, one may also find tracheoles in the sarcoplasm.

B. The Sarcoplasmatic Matrix

All muscle cells can be considered as containing a continuous aqueous fluid phase surrounded by sarcolemma, in which myofilaments, mitochondria, fat droplets, and internal membranes are present as discontinuous bodies or separate phases. This fluid matrix contains many of the soluble proteins of the muscle fiber. As seen in electron micrographs, (Figs. 34–38, 40, and 42–45), the matrix (Y) is less dense than most of the components of the fiber. This relatively light density of the matrix is evident in muscle prepared by freeze-substitution without osmium or heavy metal treatment, as well as in muscle fixed in osmic or permanganate solutions. This indicates that the native matrix should have a refractive index less than that of the myofibrils and mitochondria. Huxley and Niedergerke (1954), however, have reported that the sarcoplasm has a higher refractive index than the A band. Their interference photomicrographs do indeed show a streak-like interfibrillar component with a refractive index higher than that of the A bands. But the transverse spacing of these long streaks is rather greater than the myofibrillar diameter, and many of the streaks taper off after extending the length of only a few sarcomeres. It is evident that the structure responsible for these effects is an intermittent component of the sarcoplasm rather than the sarcoplasmatic matrix itself. It seems reasonable to attribute the effect to long chainlike accumulations of mitochondria or sarcosomes. These have the requisite properties of density and high refractive index, and are often disposed in long linear groups extending along the length of many sarcomeres, but are not found in all interfibrillar positions. But the sarcoplasmatic matrix itself is less dense than the myofibrils and has a lower refractive index (Vlés, 1911).

The matrix contains a goodly number of smallish granules scattered about in it. These are but a few tens or hundreds of Angstroms across, and are quite variable in distribution. Some regions of some muscle are almost entirely devoid of these granules, as seems to be the case in the sarcoplasmatic areas displayed in Fig. 43. But Fawcett and Selby

(1958) have found areas of sarcoplasm in heart muscle where these granules are very abundant (Figs. 37 and 45), and have brought forth evidence that they may consist of glycogen (G). This evidence hinges on the observation that areas of muscle rich in these granules give a strong periodate-Schiff reaction which is abolished by prior treatment of the tissue with amylase, and on the demonstration that areas rich in these granules are not basophilic, thus leading to the conclusion that they do not contain nucleoprotein.

Granules corresponding to the particles of Palade (1953a, 1955), which are rich in ribonucleic acid, have not been positively identified in sarcoplasm of mature muscle, although in the first few electron micrograph studies of muscle, a number of authors assumed that any cytoplasmic particles of appropriate size and density belonged to this class. But Fawcett and Selby (1958) have shown that it is incautious to make such assumptions, as error can result. At the present state of knowledge, hazard obtains when one attempts to identify small sarcoplasmatic granules in this size range, but in muscle it would appear from the work of Fawcett and Selby that most of them contain glycogen. The great abundance of glycogen granules in some areas of some muscle can be ascertained from scrutiny of Figs. 37, 40, 44, and 45. It is well known that the concentration of glycogen in muscle is variable, and can increase or decrease rapidly with varying states of nutrition, endocrine influences, exercise and state of development. Apart from glycogen granules, no definitive structural components have been identified in electron micrographs of the sarcoplasmatic matrix. It can be presumed, however, that the matrix represents an aqueous phase in which are dissolved soluble proteins such as myogen and myoglobin, and in which metabolites of smaller molecular weight might be found.

C. The Mitochondria or Sarcosomes

The mitochondria of striated muscle were depicted unmistakably and clearly by Aubert (1853). It may be that this is the first unequivocal published demonstration of mitochondria in any cell, although Aubert's paper was written long before mitochondria were recognized as distinctive cytoplasmic components generally present in cells. But even earlier, Henle (1841) described in muscle cells some small cytoplasmic granules, some of which might have been mitochondria. Kolliker (1857, 1888) first distinguished clearly between fat droplets

and mitochondria (which he called "interstitial granules"). Retzius (1890) characterized these structures further, calling them "sarcosomes"–a term still frequently used when referring to the mitochondria of muscle. It remained for Regaud (1909) and for Regaud and Favre (1909) to show that the "interstitial granules" of Kölliker and the "sarcosomes" of Retzius displayed the staining reactions of mitochondria. Later, Watanabe and Williams (1951, 1953) demonstrated that the sarcosomes or mitochondria of insect flight muscle possessed biochemical and biophysical properties similar to those of mitochondria in other cells. Palade (1952, 1953a) showed that mitochondria of muscle had characteristic ultrastructural features similar to those of mitochondria in other cells.

Muscle mitochondria are shown in Figs. 38, 41, 44, and 45. Additional detailed micrographs of striated muscle mitochondria can be found in the papers by Sjöstrand *et al.* (1958), Fawcett and Selby (1958), Bennett (1958b), Muir (1957), Andersson (1957), Moore *et al.* (1956), Sjöstrand (1956), Bennett (1955), Chapman (1954), and many others. The fine structure shows membranes and cristae in the arrangement now familiar to cytologists, and little is to be gained by describing these features anew here. The descriptions of mitochondria in smooth muscle published by Mark (1956) and by Gansler (1956, 1957) indicate that in these cells the mitochondrial structure is not exceptional.

A contrary view of muscle mitochondrial structure has been advanced by Harman (1955). Harman deemed muscle mitochondria to be comprised of "a coiled gel structure" without a membrane. This error was a consequence of faulty resolution and of specimen drift and astigmatism evident in Harman's electron micrographs. Sjöstrand (1956) and Bennett (1958b) have dealt in detail with this error. Moreover, Bennett (1958b) has discussed a further error of Harman's, wherein he confused the lipid droplets of muscle with the sarcosomes of the earlier literature.

One should comment on the distribution of mitochondria in muscle. It is evident that this varies greatly from muscle to muscle, though the mitochondria always lie outside the contractile fibrils themselves. In some muscles—for example, in the flight muscles of the domestic fowl, studied by Bennett and Porter (1953), very few mitochondria are to be found In contrast, Weinstein (1954) found great numbers of mitochondria in vertebrate heart muscle and Chapman (1954) reported

the same for insect flight muscle. Very large numbers of mitochondria were found by Robertson (1956b) and by Edwards *et al.* (1958) in the vicinity of myoneural junctions. But in general, mitochondria tend to be distributed between the myofibrils in longitudinal rows or along horizontal planes at levels of I bands, Z bands, or A-I junctions. Very large clusters are often seen at the ends of ellipsoidal nuclei and immediately under the sarcolemma.

Barrnett and Palade (1957), in a pioneering effort to develop enzymatic cytochemical approaches to the study of units of cell structure discernible with the electron microscope, found that dense crystals could be seen in mitochondria of muscle and other cells which had been incubated with succinate and tellurite. The sites of localization of these dense crystals were interpreted as indicating the activity of dehydrogenase systems near the sites of deposit. The dense crystals were found to be localized in close relation to the mitochondrial cristae.

D. The Sarcoplasmatic Reticulum, or System of Sarcotubules

1. *Historical Survey*

For over a century, there have appeared from time to time papers reporting that the interfibrillar sarcoplasm of muscle fibers contains an organized component which is often arranged with a relevance to the cross-striations of the myofibrils. Perhaps the most definitive early descriptions of this structure were those of Kölliker (1888), Cajal (1888), and Retzius (1881, 1890). Retzius spoke of this organized component as a sarcoplasmatic network. The most comprehensive treatment of this reticulum based on light microscope studies is that of Veratti (1902), who termed it the reticular apparatus of striated muscle. When Bennett and Porter (1953) encountered this component in electron micrographs, they referred to it as the sarcoplasmic reticulum, in conformity with the terminology already well-established in the light microscope literature. In later studies, Sjöstrand (1956), Andersson (1957), and Sjostrand and Andersson-Cedergren (1957) spoke of this component as "tubes," "tubules," or "sarcotubules." This term is descriptive of some aspects of this interesting sarcoplasmatic component.

The history of the discovery and gradual unfolding of knowledge of the sarcoplasmatic reticulum has been reviewed by Bennett (1955, 1956b, 1958b) and by Porter (1956). Insofar as it can be studied with

the light microscope, it was described and understood most fully by Veratti (1902). There followed a decline in comprehension of this reticulum. This decline was already evident when Heidenhain wrote his review of muscle structure in "Plasma und Zelle" in 1911. Heidenhain depicted a "cross-fiber network" (p. 518, Fig. 297), which, however, is rather diagrammatically portrayed when compared with Veratti's elegant and accurate illustrations. Mention of the sarcoplasmatic reticulum seems to have almost disappeared from that date until its rediscovery in electron micrographs by Bennett and Porter (1953), though some fragments of it appear in von Boga's (1938) illustrations. But text books, reviews (Barer, 1948; Haggqvist, 1931; Bailey, 1954; Fenn, 1945, Perry, 1956; Hamoir, 1956) and other papers dealing with muscle structure, function or biochemistry are, for the most part, devoid of any evidence of recognition of the existence of the sarcoplasmatic reticulum. It is astonishing that a structure once described as accurately and as beautifully as the reticulum was by Veratti (1902), should have so quickly become almost lost to man's knowledge.

Since Bennett and Porter revived interest in the sarcoplasmatic reticulum, much has been achieved in the way of clarifying and extending concepts of its description and significance. The subsequent efforts of Bennett (1955, 1956b, 1958a, b), Edwards and Ruska (1955), Edwards *et al.* (1956), Sjöstrand (1956), Andersson (1957), Sjöstrand and Andersson-Cedergren (1957), Porter (1956), and several others have contributed to this clarification. They have shown that the reticulum is a membranous cytoplasmic component. The membranes resemble in thickness those found in the cytoplasm of other cells, and termed by Porter the "endoplasmic reticulum" and by Sjöstrand, the "cytomembranes." In dimensions and basic architecture, the membranes of the sarcoplasmatic reticulum conform with Robertson's (1957a, b; 1958a, b) concept of the "unit membrane," which is mentioned earlier in this chapter in connection with the sarcolemma. In general, the membranes of the sarcoplasmatic reticulum are not associated with a granular or particulate component, as are those termed the α-cytomembranes of the exocrine cells of the pancreas (Sjostrand, 1956).

2. *Physiological Considerations*

Recent work by Huxley and Taylor (1955a, b) and by Andrew Huxley (1957a, b) has called attention to the existence in striated

muscle of a localized structure which can transmit a signal leading to a contraction from the sarcolemma transversely towards the interior of the muscle. In order to facilitate an understanding of the possible physiological significance of the sarcoplasmatic reticulum, it may be helpful to review briefly some of the main points in the experiments. These workers isolated striated muscle fibers of frog, crab, and lizard. To the outer surface of the sarcolemma (S) they applied the tip of a movable electrode micropipette (E) filled with a conducting solution

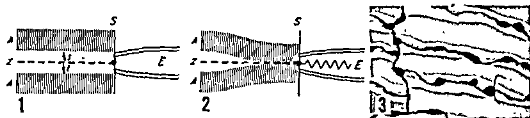


Fig. 1. Diagram of a portion of a muscle fiber of an adult frog as represented in the experiments of Huxley and Taylor (1955a). The sarcolemma (S) is represented by a vertical line to the right of the representation of two A bands and the intervening I and Z bands. A sensitive spot is represented by a black dot at the projection of the Z band to the sarcolemma. Against this sensitive spot a stimulating pipette electrode is placed, with its contained electrolyte solution in contact with the outer surface of the sarcolemma. This represents the status when no stimulating current is flowing.

Fig. 2. The same muscle fiber during the passage of a stimulating current through the electrolyte filling the pipette electrode and the sarcolemma at a sensitive spot as described by Huxley and Taylor (1955a). The two adjacent half-sarcomeres are shown as responding to the stimulus by a localized contraction extending a few microns beyond the sarcolemma into the fiber. The widths of the A bands do not change, and the proximal borders of the A bands approach each other very closely at the Z band. Sarcomeres above and below the ones represented are not caused to contract by the stimulus.

Fig. 3 Drawing of a portion of Fig. 17 from Veratti's (1902) paper, showing the sarcoplasmatic reticulum of adult frog muscle as seen in the light microscope after metallic impregnation by Veratti's method. The blackened strands of reticulum lie along the Z bands, at levels corresponding exactly to those found by Huxley and Taylor to be sensitive to localized stimulation (see Figs. 1 and 2). Figure 3 includes part of a "Nonius" unconformity in the banding of the muscle. Along the "fault line" of the unconformity is a longitudinal strand which connects the transverse strands at the Z band levels There is no evidence from Huxley's work of longitudinal conduction within the muscle fiber.

(see Figs. 1 and 4). The inside diameters of the electrodes were of the order of 2 μ. The pipettes were connected to a suitable source of a stimulating current pulse. Pulses of about $^1/_2$ sec. duration could be applied, making the interiors of the tips about 20–40 mv. negative with respect to the bath in which the fiber was immersed. The stimu-

lating currents were very much weaker than the minimum ones required for initiating propogated activity of the membrane of the fiber. A micropipette and stimulus were applied to the muscle fiber while it was under observation under a polarizing microscope. The tip of the pipette was moved about from place to place on the surface of the fiber and the response of the fiber was observed as stimuli were applied at each place.

It was found in the course of these experiments that no contractile

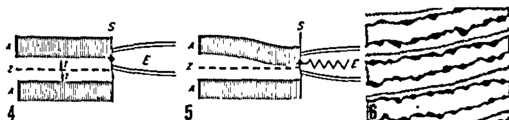


FIG. 4. This corresponds to Fig. 1, except that it represents muscle of the lizard, *Lacerta*, rather than frog. In lizard muscle, Huxley was able to elicit a localized contractile response only when the stimulating pipette electrode (E) was placed on certain sensitive spots on the sarcolemma (S) opposite the line of junction of A and I bands. The sensitive spot is indicated by a black dot on the sarcolemma. The electrolyte in the electrode (E) [illegible]

[illegible] applied through the electrode (E) to a sensitive spot on the sarcolemma (S), as demonstrated by A. F. Huxley. In this muscle, only the half-sarcomere opposite a sensitive spot responds to this type of stimulus. The localized contraction extends only a few microns onto the fiber from the stimulated spot on the sarcolemma, and is characterized [illegible] ...ding

[illegible] ...g the sarcoplasmatic reticulum of a muscle of the lizard, *Lacerta*, as seen in the light microscope after impregnation by Veratti's method. In this muscle, the reticulum appears as a series of transverse networks, each located at the levels of A-I junctions, at positions corresponding exactly to the sensitive spots found by Huxley in lizard muscle. No longitudinal strands were found by Veratti in this muscle

response could be observed when stimulating currents were applied at many places on the surface of the fiber, but when the pipette was applied to certain discrete loci, a localized contractile response could be observed (Figs. 2 and 5). In frog semitendinosus muscle fibers, the sensitive spots were found here and there along the middle of the I band, centering on a projection of the Z. Many sites at this level were insensitive, but when stimulating currents were applied at certain spots opposite the Z band, a contraction of the two adjacent half-

sarcomeres could be observed, extending into the interior of the fiber for a number of microns (see Figs. 1 and 2). The contractions in these instances were characterized by a symmetrical drawing together of the A bands of the respective two sarcomeres straddling the I band to which the stimulus was applied, with a narrowing and almost complete disappearance of the stimulated I band during the localized response. Neighboring sarcomeres were unaffected.

In the case of crab and lizard muscle fibers, sensitive spots were similarly encountered, but in these cases at the levels of junctions between A bands and I bands and not at Z band levels. When sensitive spots were activated by the pipette, the underlying half-sarcomere contracted, the contraction extending some microns into the fiber, being characterized by a reduction in the width of the adjacent half I band, and an approach of the edge of the A band to the Z (Figs. 4 and 5). The half-sarcomeres adjacent to that contracting gave no visible contractile response to the stimulus, though they were displaced somewhat by the contracting half-segment.

3. *Correlation of Structure and Function*

It is interesting to compare these physiological experiments with some of the figures from Veratti's (1902) paper, some of which have been prepared in schematic form and are presented among the figures of this chapter. Figures 7–10[1] show two longitudinal and two cross sections of several types of muscle in which the reticulum has been impregnated and drawn by Veratti. It can be seen in each example that strands of reticulum extend out from the contractile myofibrils, ending in close relation to the sarcoplasmatic surface of the sarcolemma. It would be interesting to know if the points of termination of such strands correspond to the sensitive areas of sarcolemma discovered by Huxley and his co-workers.

Further interesting points emerge if one examines and compares longitudinal views of some of the muscles depicted by Veratti with the sensitive spots and contraction patterns elicited by Huxley. It will be recalled that a localized response was obtained in frog muscle if a sensitive spot was stimulated opposite the Z. The resulting contraction

[1] Figures 7, 8, 9, and 10 are redrawn from Veratti's (1902) paper, and are selected to show Veratti's representations of connections between the sarcoplasmatic reticulum and the sarcolemma in several muscles. It is possible that the terminations of strands along the sarcolemma may correspond to the sensitive spots described by A. F. Huxley (1956).

involved nearby portions of the two adjacent half-sarcomeres (Figs. 1 and 2). Veratti's metallic impregnation of frog muscle displayed constituents of the reticulum disposed transversely at the levels of the Z bands, just at the levels at which the exploring pipette could elicit a response (Fig. 3). In a similar manner, the level of the sensitive spots of lizard muscle, detected by Huxley at the levels of A-I borders, and the resulting contraction involved only neighboring portions of the corresponding half-sarcomere (Figs. 4 and 5). Veratti's prepara-

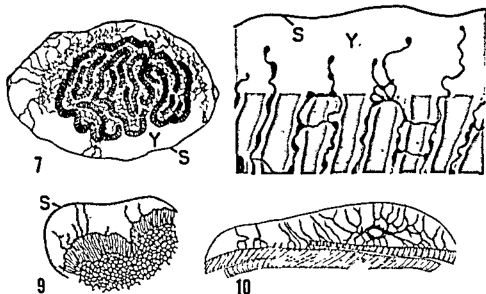


FIG. 7. This is redrawn from Veratti's Fig. 23, and represents a cross section of a dorsal fin muscle fiber of the sea horse, *Hippocampus brevirostris.* The cross sections of the myofibrils appear as convoluted ribbonlike structures embedded in a large mass of sarcoplasm (Y), surrounded by sarcolemma (S). A number of tortuous anastomosing strands of sarcoplasmatic reticulum fan out from the myofibrils and end on the inner surface of the sarcolemma.

FIG. 8. This is redrawn from Veratti's Fig. 21 and represents a longitudinal section through a muscle fiber from the red lateral line muscle of the fish, *Cyprinus carpio.* In this muscle, the transverse components of the sarcoplasmatic reticulum occur at levels of the Z bands, and from their circumferences, strands of reticulum are shown extending across the subsarcolemmatic sarcoplasm (Y) towards the sarcolemma (S). Many of these strands end in close relation to the inner surface of the sarcolemma as shown in Fig. 9.

FIG. 9. Cross section of a fiber from the same muscle represented in Fig. 8. It is redrawn from Fig. 20 of Veratti's paper The transverse networks of reticulum seen edge on in Fig. 8 here appear in plain view, showing a meshwork through whose interstices pass the myofibrils. From the circumferences of this network, strands traverse the subsarcolemmatic sarcoplasm (Y) to the sarcolemma (S).

FIG. 10 This is redrawn from Veratti's (1902) Fig. 40, which represents a longitudinal section of a

shown in Figs. 19 and 22.

tions of the reticulum of lizard muscle show a transverse component at the level of each A-I junction at precisely the levels on which sensitive spots were found by Huxley (Fig. 6). These correspondences between the elements of reticulum demonstrated by Veratti and the localization of sensitive spots and of localized half-sarcomere contractions suggest a functional relationship. It is tempting to speculate that Veratti may have demonstrated a structure capable of participating in the response to a weak stimulating localized sarcolemmatic current and in the conduction of this impulse transversely some microns into the fiber, transmitting its signal to adjacent half-sarcomeres.

Correlations of structure and function in this connection are not always as satisfying as in the examples cited above. Huxley found that crab muscle showed sensitive spots and responses similar to those illustrated in Figs. 4–6 for lizard muscle. Veratti's study of crab muscle showed transverse units of the reticulum at levels of each A-I junction, as in the lizard, and in addition, short longitudinal strands bridging each half-I band to the Z, where transverse strands were also seen (see Figs. 13 and 23). Veratti's figure suggests at first glance that in crab muscle sensitive spots might be found at the levels of the Z bands and of the A-I junctions, whereas Huxley found them only at the latter levels. Veratti's figures suggest further that a localized contraction in crab muscle might involve two adjacent half-sarcomeres straddling a Z band, as in the frog, whereas in fact it involved only one half-sarcomere, as in the lizard. Though it is not certain that the crab muscle studied by Huxley was identical, as far as reticulum disposition is concerned, with those pictured by Veratti, it seems evident that internal conduction pathways within muscle fibers cannot always be assumed to be continuous over all the structures which show mutual continuity in Veratti's diagrams.

4. *Variations in Patterns of Reticulum as Seen with the Light Microscope*

With these reservations in mind, it is of interest to glance at examples of some of the reticulum patterns depicted by Veratti, and then to compare these with the electron micrographs so far available. Much further physiological and cytological work will be necessary before the internal conducting system of muscle cells can be understood. Figure 14 shows one of Veratti's impregnations of the reticulum from the muscle of an adult white rat. The transverse component lies parallel to the Z bands, and longitudinal components connect adjacent transverse members here and

there. This can be contrasted with the example in Fig. 3, where transverse members occupy the same levels with respect to the sarcomere crossbands, but where longitudinal connections are lacking except at intersections of out-of-phase sarcomeres at "Nonius" unconformities.

Figures 15 and 16 show Veratti's examples of muscles from bat and pigeon respectively. Each of them shows a transverse component of the reticulum disposed at the levels close to the A-I junctions, corresponding to those occupied by reticular strands in the lizard muscle shown

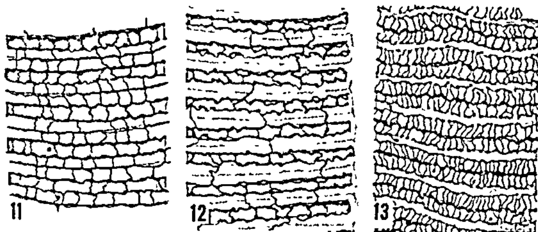


FIG. 11. This is redrawn from Veratti's Fig. 31, which represents a longitudinal section of a muscle fiber from the claw of a crayfish, *Astacus fluviatilis*, fixed in a state of relaxation. Transverse planes of reticulum are shown at levels of the Z band and of A-I junctions, with connecting longitudinal strands.

FIG. 12. *This is from Veratti's Fig. 35, representing a longitudinal section of a* limb muscle of the giant water beetle, *Hydrophilus piceus*. In this muscle, transverse networks are seen at levels of each A-I junction, with longitudinal connections traversing both A and I bands.

FIG. 13. This is from Veratti's Fig. 25, showing a diagram of a section of a muscle fiber from the leg of the crab, *Carcinus maenas*. Transverse members appear at levels of Z bands and of A-I junctions. I bands are traversed by longitudinal connecting strands of reticulum, but the A bands are not.

in Fig. 6. But whereas the transverse networks of the lizard muscle are not connected to each other longitudinally, those of the bat and pigeon muscles are connected in pairs by short longitudinal strands. But in these two muscles the pairing occurs in two different manners. In the bat muscle, the transverse components are paired by short longitudinal strands which traverse the A bands (Fig. 15), whereas in the pigeon muscle, the longitudinal strands pass across the I and Z bands as they connect the transverse networks in pairs (Fig. 16). It would be interesting to know if these two ways of pairing are reflected in differing physiological behavior of the respective muscles.

Figures 11–23[2] include several other illustrations of Veratti, depicting the sarcoplasmatic reticulum as impregnated by his method and as it appears in longitudinal section in several different types of muscles. It is not profitable at this time to speculate on correlations of such complex reticular configurations with detailed patterns of physiological conduction within the muscle fiber.

Figures 24–26 reproduce three of Veratti's figures of the sarcoplasmatic reticula of muscles of embryonic, larval, or newborn animals as

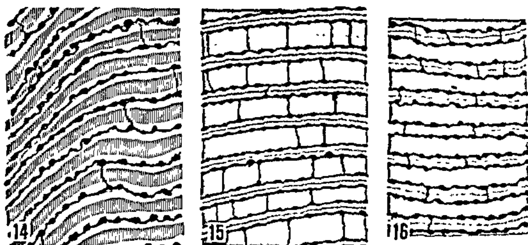


Fig. 14. This is from Veratti's Fig. 3, representing a longitudinal section of a muscle from an adult rat. Transverse nets of reticulum occur at each Z band level. These are connected here and there by short longitudinal strands extending from Z to Z.

Fig. 15. This is from Veratti's Fig. 8, which is a longitudinal view of the muscle of a bat. This resembles closely Veratti's Fig. 6, showing tongue muscle of an adult mouse. In both these muscles, transverse networks of reticulum are stretched out at each A-I junction and are connected in pairs by short longitudinal strands bridging the A bands. Connections across I bands are rare or absent in these muscles, though one is shown in the diagram.

Fig. 16. This is from Veratti's Fig. 10, representing a longitudinal view of a muscle of an adult pigeon Here also transverse networks occur at levels of the A-I junction and are paired by short longitudinal strands, but the strands traverse I bands, and not the A bands, as in the bat muscle shown in Fig. 15.

they appear in longitudinal view. It is of interest that in these young muscles there is little obvious correlation between the disposition of members of the reticulum and the cross bands of the sarcomeres of the myofibrils. Yet examples of the reticulum of muscles from adult animals of the same species show a clear transverse stratification. This suggests

[2] All these figures are redrawn from Veratti's (1902) paper to show some variations in the configuration of the sarcoplasmatic reticulum as seen in the light microscope after impregnation by his method.

that the irregular pattern of the younger forms may represent a primitive manifestation of the sarcoplasmatic reticulum. The pattern bears some resemblance to the concept of the grosser disposition of the sarcoplasmatic reticulum presented for mammalian heart muscle by Porter and Palade (1957) and reproduced in diagrammatic form in Fig. 32 of this chapter. It would be of interest to know the physiological characteristics of an irregular reticulum such as that found in juvenile, larval, and embryonic vertebrate muscle.

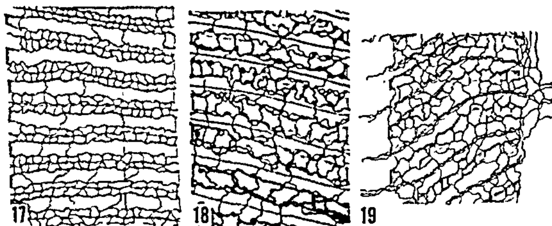


Fig. 17. This is from Veratti's Fig. 29, representing a muscle from the crab, *Carcinus maenas*, fixed in contraction. Transverse reticular planes are shown at levels of Z bands and of A-I junctions. The I bands are bridged by numerous short longitudinal strands. The A bands are bridged less abundantly by longitudinal connections.

Fig. 18. This is from Veratti's Fig. 36, representing the reticulum from the muscle fiber from a limb of the water beetle, *Hydrophilus piceus*. Rather elaborate skeins of transverse reticulum at the A-I junction levels are connected by many strands crossing the I bands and by two or three times as many crossing the A bands.

Fig. 19. This is from Veratti's Fig. 41, and represents the reticulum as drawn from a muscle fiber from the larval fly, *Gastrophilus equi*. In this preparation, one sees strands of reticulum extending out into the sarcoplasm, many from Z bands levels, though others are not clearly associated with any of the cross bands. More closely associated with the myofibrils are transverse skeins at levels of the Z bands, connected by irregular longitudinal, oblique, and transverse strands which form irregular transverse plexuses at about the levels of the A-I junctions.

The appearance of the sarcoplasmatic reticulum in cross section as it appears when studied with the light microscope in several types of muscles is shown in Figs. 7, 9, and 27–29,[3] all of which are redrawn from Veratti's paper. It is seen that the transverse components form true

[3] Figures 27, 28, and 29 show the sarcoplasmatic reticulum as it appears in plan view in cross sections of three types of muscle.

enlargements lying close to those of the set immediately neighboring. Between the saclike enlarged terminals of each set, opposite each Z band, occur rows of small vesicles resembling those between the sets in the rat muscle. In this type of muscle, elements of the reticulum encircle each myofibril completely only in the region of the M bands, where connections can be found between bracelets surrounding adjacent myofibrils. Longitudinal elements extend from the central bracelet at the levels of the M and Z bands.

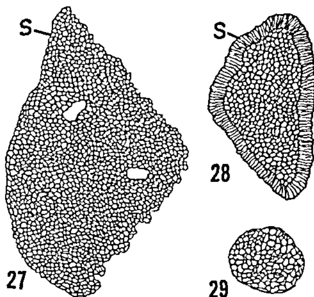


FIG. 27. This is from Veratti's Fig. 18. It shows the reticulum in a cross section of a part of a fiber from an adult frog. The sarcolemma is represented along the curved left margin. The oblique right upper and right lower margins are arbitrary edges of the drawing representing sites within the fiber. The myofibrils run at an angle normal to the drawing, and pass through the meshes of the reticulum. Each of the transverse strands occurring at levels of Z bands, as shown in longitudinal section in Fig. 3, would be represented by a transverse network as shown in this figure.

FIG. 28. This is from Veratti's Fig. 19, representing a muscle from a fish, *Cyprinus carpio*. This differs from the red lateral line muscle from the same animal, shown in Fig. 9 In the present case, large amounts of sarcoplasm outside the myofibrils are lacking, and the border of the drawing represents the sarcolemma itself. The circumferential subsarcolemmatic row of myofibrils are ribbonlike in cross section, with the long axes of the cross section arranged radially. The cross sections of myofibrils in the interior of the fiber are not specially elongated in any one direction. The strands of reticulum are blackened by the impregnation procedure and, running in the sarcoplasm, outline the cross sections of the myofibrils

FIG 29. This is from Veratti's Fig 2, showing [illegible] section in a muscle from a newborn rat (*Mus ra* [illegible] shown in longitudinal section in Fig 25. In this r [illegible] oped or differentiated, the sarcoplasmatic reticulum shows less regularity or order in structure than in the corresponding muscles of adult animals.

Fig. 32 is taken from Porter and Palade's diagram of the sarcoplasmatic reticulum as they conceive of it as arranged in rat heart muscle. In this type of muscle, there is less regularity in the disposition of the reticulum than in the other two muscles discussed here. Yet, in the rat heart muscle, there is likewise a type of organization of the reticulum into sets. Each unit or set can be considered to be a series of interconnected bracelets surrounding each myofibril, one to each sarcomere, the equator of each bracelet lying at the level of the corre-

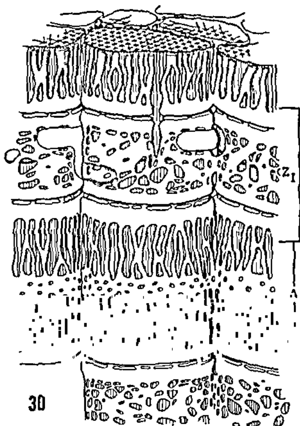


FIG. 30. This is redrawn from Text Fig. 2 in the article by Porter and Palade (1957, p. 283). It represents their concept of the geometry of the sarcoplasmatic reticulum as related to myofibrils and sarcomere bands in a muscle fiber from the sartorius muscle of the rat. Magnification: × 21,000.

sponding M band. Each bracelet has its edge at the level of the A band and consists of an irregular plexus of anastomosing tubules embracing the A band regions, from which tubular projections extend out across the I bands, terminating in flattened enlargements which lie close to the terminals of corresponding projections from the tubular plexus of the adjacent sarcomeres at the levels of the Z bands. Between these

enlarged terminals are found slender elongated vesicles arranged in rows at the level of each Z band. In this muscle alone of the three studied in detail, Porter and Palade found occasional tubulelike strands of reticulum bridging a Z band from one sarcomeric set or unit of reticulum to its neighbor in the next sarcomere.

It must be emphasized that in all three of the muscles studied, Porter and Palade found each set or unit of reticulum to be connected to its neighbors transversely, so that a continuity of reticulum across the en-

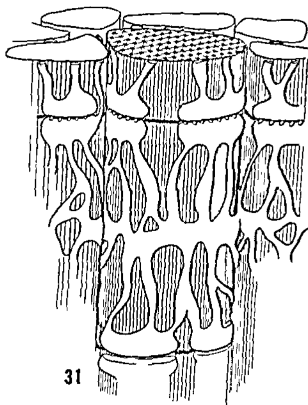


FIG. 31. This is redrawn from Text Fig. 1 of Porter and Palade (1957, p. 279). It represents their formalized concept of the sarcoplasmatic reticulum as related to sarcomere bands and myofibrils as it appears in muscle fibers in myotomes of the larval salamander, *Amblystoma punctatum* Magnification: × 24,000.

tire fiber occurred at each level represented by a unit or set of reticulum. These anastomoses between the bracelets or units surrounding individual myofibrils are represented in Figs. 30, 31, and 32.

Another important detail of the system of sarcoplasmatic reticula, applicable to all three muscles and to others as well, is a special relation between units of reticulum and the sarcolemma. Some examples are

represented in the electron micrographs of Figs. 38 and 42 and in the diagrams shown in Figs. 7, 8, 9, 10, and 33. Connections between the sarcoplasmatic reticulum and the sarcolemma often appear in electron micrographs as irregular rows of elongated or spheroidal vesicular profiles extending transversely from a region of the reticulum to the sarcolemma at the level of a Z band. Frequently caveolae and subsarcolemmatic vesicles characterize the sarcolemma at such sites. One published picture by Porter and Palade (1957) shows that the relationship

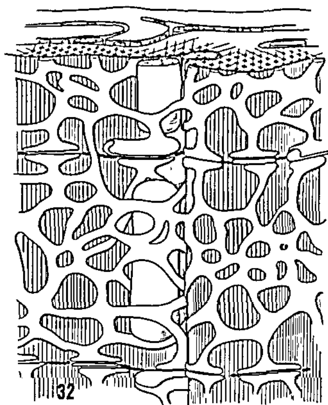


FIG. 32. This is redrawn from Text Fig. 3 of Porter and Palade's paper (1957, p. 288). It represents their concept of the sarcoplasmatic reticulum as invisioned in the cardiac muscle of the rat. Magnification: approx. × 22,000.

between the sarcoplasmatic reticulum and the sarcolemma can show interesting morphological specialization in certain cases. A diagram drawn from Fig. 14 of Porter and Palade's (1957) important paper, is presented herewith in Fig. 33. It can be seen that the sarcolemma is here indented at the level of the Z band. Closely applied to the inner surface of the sarcolemma, but separated from it by about 150 A., are membranous structures which represent specializations in extensions

of the sarcoplasmatic reticulum to the sarcolemma. In the profile evidenced in the micrograph, tubular members of the reticulum approaching the sarcolemma at the level of a Z band appear to form a pair of lobelike projections which apply themselves to the inner surface of the sarcolemma. The full three-dimensional shape of these structures cannot be deduced from Porter and Palade's single micrograph. Knowledge of this may have to await reconstruction from serial sections. It is tempting to think this might represent one of the sensitive sarcolem-

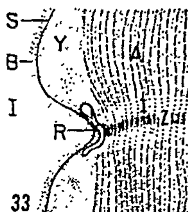


Fig. 33. This is a schematic tracing taken from Plate 94, Fig. 14, of the paper by Porter and Palade (1957). It represents a tracing of an electron micrograph showing the margin of a cardiac muscle fiber of a rat. The sarcolemma (S) and basement membrane (B) separate subsarcolemmatic sarcoplasm (Y) from the intercellular space(I). A myofibril with A, I, and Z bands is indicated. Profiles of membranes of the sarcoplasmatic reticulum (R) extend out from the Z band, forming two oblique flattened vesicles, each of which presents a surface lying close to the inner surface of the sarcolemma. One is tempted to speculate that this might represent an area where, during the passage of a propagated impulse along the sarcolemma, a signal might be picked up and transmitted to myofibrils within the fiber. *Magnification:* approx. × 29,000.

matic spots demonstrated by A. F. Huxley (1957b), but direct evidence as to its functional significance is lacking. Nevertheless, it may be profitable to consider such morphological relationships as this in attempting to formulate experiments or models designed to improve understanding of the mechanism of conduction of a signal from an excited region of the sarcolemma to myofibrils in the fiber.

6. *Correlation of Light and Electron Microscope Findings*

It is pertinent to ask what components of the sarcoplasmatic reticulum evidenced in electron micrographs are represented in the impregnations of Veratti. In general, Veratti's impregnations tend to

portray a more simple pattern than appears in electron micrographs, or in the diagrams derived from them, examples of which appear in Figs. 30, 31, and 32. One gains the impression that Veratti's impregnation method was not consistent, and that some of his preparations yielded a more complete demonstration of the reticulum than others. For example, those diagrammed in Figs. 3, 6, 14, 15, and 16 show in the main transverse networks at the levels of either Z Band or A-I junction. If one then examines electron micrographs or the reconstructions shown in Figs. 30, 31, and 32, it seems apparent that in these instances, Veratti may have studied preparations in which metal was deposited primarily in the rows of slender vesicles evident in the electron micrographs between transverse sets of reticulum at levels of the H band or A-I junction (Fig. 30) or Z band (Figs. 31 and 32). In two of these cases, as illustrated in Figs. 3 and 6, there is an interesting correspondence between the sensitive region, the localized contraction, the location of the metal deposits in Veratti's preparations, and the row of small vesicles seen between the sets or units of reticulum in electron micrographs. This suggests that the rows of small vesicles seen in electron micrographs between enlargements of terminal members of sets of reticulum related to sarcomere units may have an important role in transmitting an excitatory signal to the contractile units and may have a preferential capacity to bind metal in the course of Veratti's impregnation procedure. Yet it is evident that most of the sarcoplasmatic reticulum is not demonstrated in these particular examples of Veratti's work.

But many of Veratti's preparations reveal a much more elaborate skein of structures, some of them corresponding in complexity to the elaborate diagrams of Porter and Palade (Figs. 30, 31, and 32). Examples of some of Veratti's preparations of this type are reproduced in Figs. 20–23. Demonstrations of reticulum of intermediate complexity are presented from Veratti's work in Figs. 11–13 and 17–19. If one examines carefully the specimens portrayed in Figs. 20–23, one gains the impression that in these examples much, if not all, of the sarcoplasmatic reticulum is demonstrated in gross outline. But concealed by the resolving limitations of the light microscope may be discontinuities which might separate the members of the reticulum in one set from corresponding projections from the set in the next sarcomere. Such matching projections are diagrammed in Figs. 30, 31, and 32. This condition may have deceived Veratti into thinking that the sarcoplas-

matic reticulum was, in many types of muscle, continuous longitudinally over many sarcomeres. The electron micrographs, however, have shown that this appearance may be misleading, and that discontinuities too narrow to be detected by the light microscope may occur. Caution is signaled by certain other inconsistencies between Veratti's pictures and electron micrographs. Veratti's (1902) Fig. 3, part of which is reproduced in this chapter as Fig. 4, shows his display of the sarcoplasmatic reticulum in an unspecified muscle of an adult white rat. In this and in Veratti's Fig. 5, which likewise shows a rat muscle, the transverse networks of reticulum impregnated by his method appear at levels of Z bands. In Fig. 30 and Fig. 41 of this chapter, which are taken from the electron microscope study of Porter and Palade (1957), the reticulum of rat sartorius muscle is depicted. Here the rows of small intermediary vesicles comprising the central member of the triads occur at levels of the A-I junctions or N bands, and not at levels of the Z bands, where Veratti's studies showed a definite structure. The electron micrographs of rat sartorius muscle show no feature which can reasonably be correlated with the network shown by Veratti. However, Veratti's Fig 6 shows a muscle fiber from the tongue muscle of another animal of the same genus, *Mus decumanus.* Here transverse networks are shown at levels of the N bands or A-I junctions, corresponding in position to the rows of intermediary vesicles demonstrated by Porter and Palade in rat sartorius muscle fibers.

It is not certain that these apparent inconsistencies between Veratti's portrayal of the reticulum of rat muscle and the electron micrographs of Porter and Palade are real. Veratti did not state what muscle of the rat is represented in his drawings. It is possible that it was not the rat sartorius muscle, or that the muscle fiber represented has an arrangement of the sarcoplasmatic reticulum dissimilar to that of the sartorius muscle of the adult rat. Moreover, within a single muscle, two or more types of fibers are often found (Knoll, 1891; Bullard, 1912; Denny-Brown, 1929; Wachstein and Meisel, 1955). No electron microscope study published up to the present has dealt with the sarcoplasmatic reticulum in different types of fibers within the same muscle. It may be that the configuration reported by Porter and Palade for the reticulum of rat sartorius muscle actually represents the patterns for only one type of fiber in that muscle, and that different types of fibers within a single muscle may reveal differing configurations of the sarcoplasmatic reticulum.

Only further work can dispel the uncertainties now existing with

respect to the variations to be expected in the sarcoplasmatic reticulum among fibers of the same muscle and among fibers from different muscles. Further work is also needed to clarify questions relating to possible changes in the sarcoplasmatic reticulum with contraction, fatigue, degeneration, and development. Variations due to these factors may also be clouding our vision at the present time.

The evidence derived from the electron microscope, then, permits us to interpret Veratti's preparations usefully with the following three reservations in mind: First, the pattern of reticular structure may vary from animal to animal, from muscle to muscle within the same animal, and even may differ for differing types of fibers within the same muscle. Second, Veratti's methods do not always demonstrate the whole sarcoplasmatic reticulum, but may at times reveal only portions of it. Third, there may be discontinuities in the reticulum which are too narrow to be detected by the light microscope. Thus structural continuity between strands of the reticulum cannot always be assumed even though an appearance of continuity may be evident when impregnated specimens are studied with the light microscope. But within the limitations of their respective methods, it appears that the methods of Veratti and of electron microscopy provide valuable information relating to the organization of the sarcoplasmatic reticulum. This information is now finding useful physiological implementation.

7. *Fine Structural Details of the Sarcoplasmatic Reticulum*

From the micrographs reproduced in Figs. 38 and 41–45, it is evident that the strands of reticulum represented as black lines in Veratti's illustrations are actually membrane-bound tubules, channels, bullae, and cisternae upon which the impregnating metal has been deposited. Electron micrographs show that the membranes bounding the channels of the reticulum are similar in structure to the sarcolemma and to other examples of the "unit membrane" described by Robertson (1957a, b; 1958a, b) and discussed in earlier passages in this chapter. One of Robertson's high resolution electron micrographs of elements of the sarcoplasmatic reticulum appears in Fig. 40.

Although these membranes in cross section display two peaks in density separated by an interval of lesser density, and appear to be about 75–100 A. thick overall, this similarity in structure to the sarcolemma, to cytoplasmatic membranes in general, to those of mitochondria, and to the nuclear envelope should not lead to the conclusion

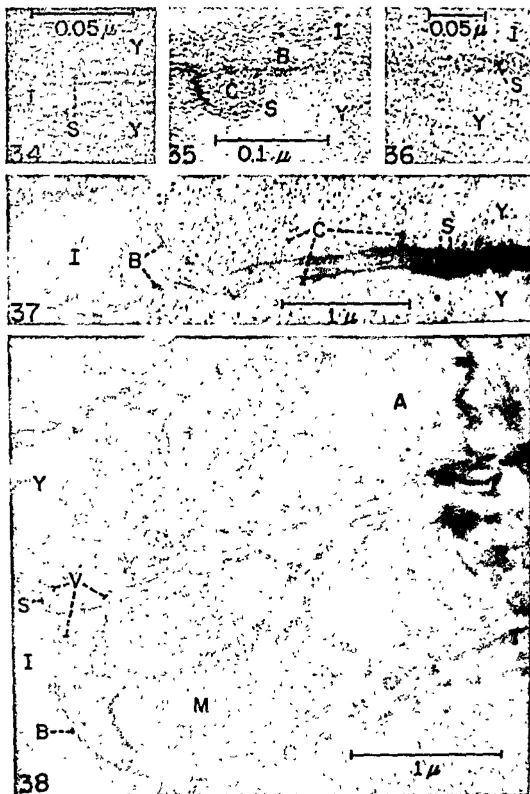

0.05 μ
Y
I
S
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34
I
B
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S
Y
35
0.1 μ
0.05 μ
I
S
Y
36
I
B
C
S
Y
Y
37
1 μ
A
Y
V
S
I
M
B
38
1 μ

PLATE I

that all of these membranes are really identical in structure. Differences in physiological properties of various membranes betray differences in molecular structure which are not evident in electron micrographs, which can at best only sketch out in rough form the geometry of the framework upon which the molecular components are arranged. Thus it is possible that among various components within the sarcoplasmatic reticulum, considerable and significant variations in structure might be present at levels of organization not resolved by the electron microscope. But even with the electron microscope, certain differences in density are discernible if some elements of the sarcoplasmatic reticulum are compared with others. These will be discussed in later passages of this chapter.

The membranes defining the borders of the sarcoplasmatic reticulum

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Fig. 34. Electron micrograph by Dr. J. D. Robertson, showing unit membrane structure of sarcolemma of two adjacent smooth muscle cells. Permanganate fixation, Araldite embedding. Y designates the cytoplasm of the two muscle cells, while I indicates an intercellular space perhaps 100 A wide. This intercellular space is more dense than the sarcoplasm. Magnification somewhat uncertain but probably approx. × 400,000.

Fig. 35. Electron micrograph by Dr. J. D. Robertson, showing unit membrane and caveola of the sarcolemma of a striated muscle cell. Permanganate fixation and Araldite embedding. The unit membrane, with its two dense components and its less dense central lamina, is here indented into the cytoplasm to form a small pit or depression or caveola about 300 A. deep and 400 A. across. In the intercellular space just outside the sarcolemma is the basement membrane, B. Magnification: approx. × 240,000.

Fig. 36. Electron micrograph by Dr. J. D. Robertson, of the sarcolemma of a striated muscle cell, showing characteristic unit membrane structure. Permanganate fixation and Araldite embedding. Magnification. × 250,000.

Fig. 37. Electron micrograph by Professor D. W. Fawcett and Dr. C. C. Selby of heart muscle from the atrium of a heart of a turtle. Osmic fixation and methacrylate embedding. The sarcoplasm (Y) is punctuated with glycogen granules and is separated from the intercellular space (I) by basement membrane (B) and sarcolemma (S). The sarcolemma displays many caveolae (C). Nearby are vesicles which show no membranous connection with the sarcolemma. Magnification: × 28,000

Fig. 38. Electron micrograph by Professors K. R. Porter and G. E Palade, of heart muscle fiber of a rat. Osmic fixation and methacrylate embedding. In the lower left corner is intercellular space (I). Adjacent to the sarcolemma (S) is the extracellular basement membrane (B). The sarcoplasmatic reticulum (R) is conspicuous and is cut in some places so that extensive stretches lie in the plane of the section. The triad arrangement (T), which betokens a special connection between reticulum and myofibril, can be recognized at the level of the Z bands in this type of muscle. Near the vesicles designated (V), the reticulum comes close to the sarcolemma and appears to enter into close relation to it One can speculate that at this point an

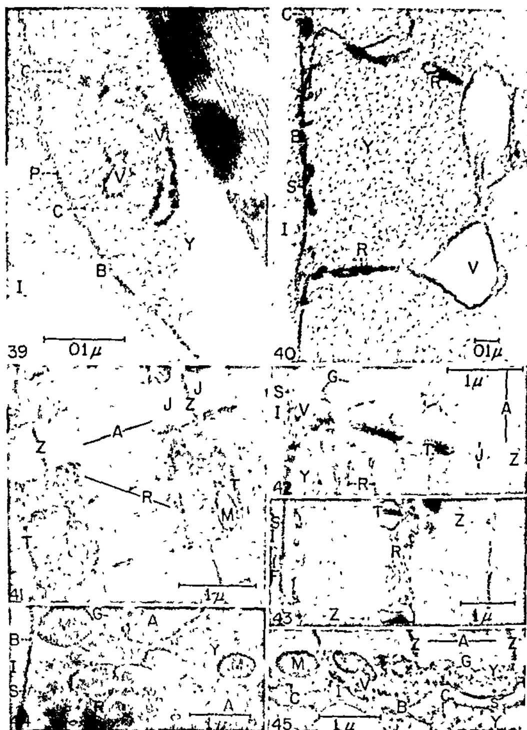


PLATE II

[illegible]
material (B) close to the plasma membrane (P) of the Schwann cell. Two caveolae (C) indent the plasma membrane into the Schwann cell cytoplasm (Y). Two profiles identified as vesicles (V) can be seen in the cytoplasm. It is possible that serial sections would have revealed one or both of them to be continuous with a caveola. Myelin [illegible] e charac- [illegible] 175,000.
[illegible] f a verte- [illegible] (B), and [illegible] vaded by strands of reticulum (R) connected to large vesicles (V), which probably represent cisternae of the sarcoplasmatic reticulum. Note that the sarcolemma is punctuated by caveolae (C) and by small vesicles nearby. The horizontal strands of reticulum a[illegible] :tween the sarcoplasmatic re[illegible]

[illegible] s K. R. Porter and G. E. Palade of a section taken from the sartorius muscle of a rat. Portions of six myofibrils are shown, with several of the crossbands of one labeled. Several mitochondria (M) also appear. Of chief interest here are elements of the sarcoplasmatic reticulum (R). The plane of section has grazed two myofibrils tangentially in such a way as to show to advantage some features of their relation to the reticulum. The letter T designates one of several "triads," or combines of paired cisternae with intermediary vesicles, all showing a special relation to the myofibrils. In this muscle two triads occur with each sarcomere at levels of junctions between A and I bands. Magnification: × 17,000.

[illegible] membrane and resemble those comprising the central vesicular component of the triads. The bulbous symmetrical outer cisternae of the triads show clearly their continuity with tubular portions of the sarcoplasmatic reticulum. In this muscle, the triad occurs one to a sarcomere at the level of the Z bands. Magnification: × 17,000.

Fig. 43. An electron micrograph supplied by Professors K. R. Porter and G. E. Palade showing reticulum and triads in a muscle of a larval *Amblystoma punctatum*. This figure shows to advantage a segment of reticulum (R) connecting two triads (T), and forming a skein of tubules meeting in a belt around each myofibril at the level of the M band. Magnification × 12,000

Fig. 44. An electron micrograph supplied by Professors K. R. Porter and G. E. Palade showing a transverse but slightly oblique section through a muscle of a larval *Amblystoma*. Myofibrils are seen in cross section, most of them cut through the bands (A). The sarcoplasmatic reticulum (R) is cut tangentially in one area, revealing its membranous, tubular nature and its relation to the cisternal enlargements at the triad Magnification × 13,000.

[illegible] respective basement membranes (B). Each sarcolemma is indented with nu[illegible] (G) and mitocho[illegible] the upper cell. A [illegible] × 11,000.

enclose spaces which are structureless at resolutions so far achieved with the electron microscope. It is presumed that the contents enclosed by the membranes consist of an aqueous proteinaceous fluid containing dissolved ions and perhaps small organic molecular components. But no chemical studies of its contents have been reported, and it seems that difficulties would confront one attempting to obtain samples sufficiently large for analysis. It is evident, however, that the contents of the reticulum comprise an aqueous phase separated by a lipid containing phase boundary residing in the membranes from the aqueous phase of the rest of the sarcoplasm. There is no reason to think its ionic content would be identical with that of the sarcoplasmatic matrix. If the ionic content does differ from that of the matrix, an electrical potential difference might exist between the two compartments. Such a voltage gradient would not be detected by present microelectrode techniques, and attempts to determine if such gradients exist would not be easy.

Each transverse set or unit of the reticulum, then, contains, by means of its membranes, a quantity of fluid which is walled off, not only from the sarcoplasmatic matrix in general, but also entirely or almost entirely from the corresponding fluid in the neighboring sets. Thus the reticulum defines a series of intrareticular or intratubular fluid compartments extending continuously transversely across the fiber, one or two such compartments being present for each sarcomere. Each compartment consists of elaborately anastomosing tubules and channels of varying caliber, tending to be flattened in planes tangential to the borders of the myofibrils.

Along lines where the border or edge of one set or unit of reticulum contacts its neighbor of the same myofibril, there occurs an interesting pattern of morphological specialization which involves, among other factors, relationships between the reticulum and the myofibrils which are not found elsewhere. These relationships are worthy of attention as possible sites at which excitatory impulses or substances may be conducted or transmitted to the myofibril. At present we have no knowledge of the physiological significance of these sites and only partial knowledge of the structure. For this we depend almost entirely on the study by Porter and Palade (1957), to which extensive and grateful reference has already been made. Examples of electron micrographs of sections through these specialized sites are presented in Figs. 38 and 41–43 (T). From these illustrations, from other figures in Porter and Palade's paper, and from Figs. 30, 31, and 32, it can be seen that at the levels of the Z bands in some muscles and at the levels near the A-I

junctions in others, the two adjacent sets or units or sarcoplasmatic reticulum which border each other present paired sausage-like or bullous dilatations, enlargements, or cisternae, which abut against their opposite fellows. Between the paired apposed enlargements are arrayed rows of small intermediary vesicles 200-300 A. across in their shortest diameter. This combination of apposed pairs of bullous cisternal dilatations and intermediary vesicles is spoken of as a "triad" by Porter and Palade (1957).

The membranes comprising the members of the triad show some variations in density from the usual pattern seen in cytomembranes in electron micrographs of sections of osmic-fixed material. Thus the side membranes of the bullous terminal enlargements are somewhat less dense than most of the membranes of the sarcoplasmatic reticulum, whereas the apposed membranes of the terminal cisternae and the membranes defining the small interposed vesicles are usually more dense than the standard. Such variations in density, as well as the association of bullous cisternae with small dense vesicles, are reminiscent of features of the Golgi membranes of many cells (Dalton and Felix, 1953, 1954; Sjöstrand and Hanzon, 1954a, b). But it must be remembered that typical Golgi bodies and membranes have been demonstrated elsewhere in striated muscle cells, characteristically in the cytoplasm in the vicinity of the poles of ellipsoidal nuclei (Luna, 1911; Fawcett and Selby, 1958).

Porter and Palade (1957) have discerned some interesting features associated with the small intermediary vesicles of the triads. In cross sections so located that the section includes the intermediary vesicles, one can see fingerlike projections extending into each myofibril from the region of the interposed vesicles around the periphery of the fibril. These projections extend in only a short distance, not further than the most circumferential two or three rows of myofilaments. Many details of these projections remain obscure. In basic structure, the projections appear to be bound by unit membranes enclosing elongated, curved sausage-like vesicles perhaps 200 A. in diameter. The inner terminals of these projections presenting to the interior of the myofibrils do not show very clearly, nor is the relationship of these inward projections to the interposed vesicles unambiguously demonstrated in any of the micrographs so far published. Because of the possibility that these sausage-like projections might have a role in transmitting or injecting an excitatory substance into the myofibril, further information relating to their structure and relationship would be very welcome.

enclose spaces which are structureless at resolutions so far achieved with the electron microscope. It is presumed that the contents enclosed by the membranes consist of an aqueous proteinaceous fluid containing dissolved ions and perhaps small organic molecular components. But no chemical studies of its contents have been reported, and it seems that difficulties would confront one attempting to obtain samples sufficiently large for analysis. It is evident, however, that the contents of the reticulum comprise an aqueous phase separated by a lipid containing phase boundary residing in the membranes from the aqueous phase of the rest of the sarcoplasm. There is no reason to think its ionic content would be identical with that of the sarcoplasmatic matrix. If the ionic content does differ from that of the matrix, an electrical potential difference might exist between the two compartments. Such a voltage gradient would not be detected by present microelectrode techniques, and attempts to determine if such gradients exist would not be easy.

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this fish, very large amounts of fat are stored in the muscle during the feeding in salt water prior to the spawning migration. This fat is used as fuel during the ascent up the rivers. The fat accumulates in large groups of droplets under the sarcolemma and in rows of smaller droplets between the myofibrils. This fat is largely used up before spawning occurs. Considerable differences occur in fat distribution in the deep pink swimming muscles as compared to the dark superficial lateral line muscles.

Fat droplets have been seen in electron micrographs of striated muscle. Some of the early observations were made by Harman (1955), who spoke of the lipid bodies as "sarcosomes" and who failed to recognize their lipid nature.

Intrasarcoplasmatic lipid droplets, as seen in the electron microscope, often are not circular in profile, but display irregular contours. Frequently they are quite dense in osmic-fixed material, apparently as a consequence of binding osmium compounds. They are found with the electron microscope just where one would expect them on the basis of light microscope studies. Some typical examples appear in Fawcett and Selby's (1958) Figs. 1 and 27, in Muir's (1957) Fig. 8, and in Moore and Ruska's (1957) Fig. 12.

G. Tracheoles

In many insect muscles, fine tracheolar branches are found in close relation to the sarcolemma and deep in the fibers, running in the sarcoplasm between the myofibrils. Descriptions of these components of insect striated muscle as seen in the electron microscope are meager. No definitive description of intrasarcoplasmatic tracheoles in insect muscle as seen in the electron microscope has yet appeared. Some casual observations on their structure appear in the paper by Chapman (1954), and an abstract mentioning some details of the fine structure of such tracheoles was presented by Bennett (1953). Edwards and Ruska (1955) and Edwards *et al.* (1958) present some pictures of tracheoles associated with the external surface of the sarcolemma of wasp leg muscle and within the sarcoplasm. But detailed knowledge of the fine structure of insect muscle tracheoles must await further studies.

IV. NUCLEI

The nuclei of striated muscle fibers do not deserve extended treatment in this chapter. The localization of nuclei in various types of

E. The Golgi Membranes

Until recent years, there has been considerable confusion in the literature relating to the nature of the Golgi apparatus of striated muscle cells. A review of this topic is available in the paper by Eastlick (1937). A number of different structures in muscle cells which could be impregnated with osmic acid or with silver, and which appeared black with the light microscope, have been called the equivalent of the Golgi bodies of muscle. With the clarification of the nature of the Golgi bodies through the work of Dalton and Felix (1953, 1954) and Sjöstrand and Hanzon (1954a, b), the characteristic membranes and configurations of the Golgi region have permitted this cytoplasmic component to be identified in electron micrographs of many types of cells. Fawcett and Selby (1958) have published excellent pictures demonstrating this component in heart muscle cells. As mentioned earlier in this chapter, characteristic Golgi membranes can be found in the cytoplasm at the poles of the ellipsoidal nuclei of muscle cells. Figure 27 from Fawcett and Selby's paper provides a good example. In the light of the electron microscope findings, it seems evident that a correct interpretation of the Golgi apparatus of striated muscle can be attributed to Luna (1911), whose elegant impregnations and careful light microscope studies show the Golgi apparatus just where it is found in electron micrographs.

F. Sarcoplasmatic Lipid Inclusions

Kolliker in 1857 described fat droplets in striated muscle fibers and distinguished them from mitochondria, which he called "interstitial granules." There have been several subsequent descriptions of fat in muscle, of which some of the most elegant are by Bullard (1912, 1916) and by Greene (1913), based on light microscope studies. Both of these authors stress that fat droplets are normal components of the sarcoplasm of many muscles, and that the presence of fat is not to be construed, in itself, as evidence of degeneration. These authors also point out that sarcoplasmatic fat content and disposition varies considerably, not only from muscle to muscle, but among fibers of different types within the same muscle and with different physiological states within the same muscle fiber.

Some particularly striking variations in intramuscular fat are described by Greene (1913) in the king salmon, *Onchorhynchos kisutch*. In

Definition of Letters Used in Figures

A A band

B basement membrane

C caveola intracellularis

E stimulating pipette electrode

F collagen (or reticulum) filaments

G glycogen

I intercellular space

J I band

L lamellae of myelin

M mitochondria

P plasma membrane

R sarcoplasmatic reticulum

S sarcolemma

T triad (of Porter and Palade)

V vesicle

Y cytoplasmic (or sarcoplasmatic) matrix

Z Z band

References

Alksne, J. F. (1959). *Quart. J. Exptl. Physiol.* **44**, 51-66.
Andersson, E. (1957). *Proc. Stockholm Conf. Electron Microscopy* 1956, 208.
Aubert, Dr. (1853). *Z. wiss. Zool.* **4**, 388.
Bailey, K. (1954). *In* "The Proteins" (H. Neurath and K. Bailey, eds.), Vol. II, Pt. B, Chapt. 24. Academic Press, New York.
Barer, R. (1948). *Biol. Revs. Cambridge Phil. Soc.* **23**, 159.
Barrnett, R. J., and Palade, G. E. (1957). *J. Biophys. Biochem. Cytol.* **3**, 577.
Bear, R. S., and Schmitt, F. O. (1936). *J. Opt. Soc. Am.* **26**, 206.
Bear, R. S., Palmer, K. J., and Schmitt, F. O. (1941). *J. Cellular Comp. Physiol.* **17**, 355.
Bennett, H. S (1953). *Anat. Record* **115**, 282.
Bennett, H. S. (1955). *Am. J. Phys. Med.* **34**, 46.
Bennett, H. S. (1956a). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 99.
Bennett, H. S. (1956b). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 171.
Bennett, H. S (1958a). Neurology **8**, 65.
Bennett, H. S. (1958b). *In* "Frontiers of Cytology" (S. L. Palay, ed.), Chapt. 12. Yale Univ. Press, New Haven, Connecticut.
Bennett, H. S., and Porter, K. R. (1953). *Am. J. Anat.* **93**, 61.
Bowman, W. (1840). *Phil. Trans. Roy. Soc. London* **130**, 457.
Bullard, H H. (1912). *Am. J. Anat.* **14**, 1.
Bullard, H. H. (1916). *Am. J. Anat.* **19**, 1.
Cajal, S. R. (1888). *Intern. Monatsschr. Anat. Physiol.* **5**, 205.
Chapman, G. B. (1954). *J. Morphol.* **95**, 237.
Chapman-Andresen, C., and Holter, H. (1955). *Exptl. Cell Research* **3** *Suppl.*, 52.
Chapman-Andresen, C , and Prescott, D. M. (1956). *Compt. rend. trav. lab. Carlsberg Sér. chim.* **30**, 57.
Coons, A. H. (1953). *In* "Th[illegible] tibody Response" (A. W. Pappenheimer[illegible] York.
Dalton, A. J., and Felix, M[illegible]

striated muscle cells has been thoroughly explored with the light microscope, and the electron microscope cannot be expected to contribute significantly to this matter. A number of papers include satisfactory electron micrographs of nuclei in striated muscle cells of various kinds. Among these papers are those of Bennett and Porter (1953), Chapman (1954), Lindner (1957), Moore and Ruska (1957), and Fawcett and Selby (1958). It seems evident that the fine structure of nuclei of striated muscle cells is not significantly different from that of nuclei in other unspecialized cells. Individual chromosomes have not been recognized in muscle cell nuclei. These nuclei are surrounded by an envelope consisting of two unit membranes, the outer member of which may be continuous with cytomembranes in the perinuclear sarcoplasm or those of the sarcoplasmatic reticulum (Porter and Palade, 1957). The envelope of striated muscle cell nuclei is likewise endowed with "pores" resembling those described in relation to other nuclei. The description and concepts of Watson (1955) can be regarded as applicable, in general, to nuclei of the muscle cells in question.

V. CONCLUDING REMARKS

Most of the studies of muscle so far reported in the literature have dealt with the contractile mechanism of muscle. This group of studies includes those relating to the proteins comprising the contracting portion of muscle (the myofilaments and myofibrils), the structure of myofilaments and myofibrils, and the coupling mechanisms which might render available the bond energy of ATP for the contractile process. A second major effort has gone into the characterization of the excitation of muscle cells by nerve impulses or by direct stimulation of the sarcolemma.

This chapter, however, deals with structural aspects of muscle which have received relatively little attention in recent years. It deals with the fueling system of muscle and with structures which support, control, regulate, and excite the contractile material itself. These topics, having been neglected in the past, are worthy of special attention in the future. One can hope for rapid advance in our knowledge of the structure and function of portions of muscle not directly involved in the contractile process. This knowledge, along with a satisfactory acquaintance with the myofilaments and myofibrils, will be necessary before one can achieve a comfortable understanding of the structure and function of striated muscle.

Odland, G. F. (1958). *J. Biophys. Biochem. Cytol.* **4**, 529.
Odor, D. L. (1956). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 105.
Palade, G. E. (1952). *Anat. Record* **114**, 427.
Palade, G. E. (1953a) *J. Histochem. and Cytochem.* **1**, 188.
Palade, G. E. (1953b). *J. Appl. Phys.* **24**, 1419.
Palade, G. E. (1955). *J. Biophys. Biochem. Cytol.* **1**, 59.
Palade, G. E. (1956). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 85.
Perry, S. V. (1956). *Physiol. Revs.* **36**, 1.
Péterfi, T. (1913). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **83**, 1.
Porter, K. R. (1956). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 163.
Porter, K. R., and Palade, G. E. (1957). *J. Biophys. Biochem. Cytol.* **3**, 269.
Reed, R., and Rudall, K. M. (1948). *Biochim. et Biophys. Acta* **2**, 19.
Regaud, C. (1909). *Compt. rend.* **149**, 426.
Regaud, C. and Favre, M. (1909). *Compt. rend.* **148**, 661.
Retzius, G. (1881). *Biol. Untersuch.* **1**, 1.
Retzius, G. (1890). *Biol. Untersuch.* (N. F.) **1**, 51.
Robertson, J. D. (1955). *J. Biophys. Biochem. Cytol.* **1**, 271.
Robertson, J. D. (1956a). *J. Biophys. Biochem. Cytol.* **2**, 369.
Robertson, J. D. (1956b). *J. Biophys. Biochem. Cytol.* **2**, 381.
Robertson, J. D. (1957a). *J. Biophys. Biochem. Cytol.* **3**, 1043.
Robertson, J. D. (1957b). *J. Physiol. (London)* **140**, 58.
Robertson, J. D. (1958a). *Anat. Record* **130**, 440.
Robertson, J. D. (1958b). *J. Biophys. Biochem. Cytol.* **4**, 349.
Ruska, H. (1954). *Z. Naturforsch.* **9b**, 358.
Schiebler, T. H. (1953). *Z. Zellforsch. u. mikroskop. Anat.* **39**, 152.
Schmitt, F. O., and Bear, R. S. (1939). *Biol. Revs. Cambridge Phil. Soc.* **14**, 27.
Schwann, T. (1839). "Mikroskopische Untersuchungen uber die Uebereinstimmung in der Struktur und dem Wachsthum der Thiere und Pflanzen," Reimer, Berlin.
Sjostrand, F. S. (1956). *Intern. Rev. Cytol.* **5**, 455.
Sjostrand, F. S., and Andersson-Cedergren, E. (1957). *J. Ultrastructure Research* **1**, 74.
Sjostrand, F. S., and Hanzon, V. (1954a). *Exptl. Cell Research* **7**, 415.
Sjostrand, F. S., and Hanzon, V. (1954b). *Experientia* X/9, 367.
Sjostrand, F. S., Andersson-Cedergren, E., and Dewey, M. M. (1958). *J. Ultrastructure Research* **1**, 271.
Veratti, E. (1902). *Mem. reale Inst. Lombardo* **19**, 87.
Vlés, F. (1911). Thèses résentées à la Faculté des Sciences de l'Université de Paris, Paris, A Hermann and Sons, Paris.
Von Boga, L. (1938). *Z. Zellforsch. u. mikroskop. Anat.* **27**, 568.
Wachstein, M., and Meisel, E. (1955). *J. Biophys. Biochem. Cytol.* **1**, 483.
Watanabe, M. I., and Williams, C. M. (1951). *J. Gen. Physiol.* **34**, 675.
Watanabe, M. I, and Williams, C. M. (1953). *J. Gen. Physiol.* **37**, 71.
Watson, M. L. (1955). *J. Biophys. Biochem. Cytol.* **1**, 257.
Weinstein, H. J. (1954). *Exptl. Cell Research* **7**, 130.
Wissig, S. L. (1958). *Anat. Record* **130**, 467.
Wotton, R M., and Mosti, M. E. (1955). *Anat. Record* **122**, 39.
Yamada, E. (1955). *J. Biophys. Biochem. Cytol.* **1**, 445.
Zetterqvist, H (1956). Thesis. Dept. Anat. Karolinska Inst. Stockholm, Aktiebolaget Godvil, Stockholm.

Dalton, A. J., and Felix, M. D. (1954). *Symposium, 8th Intern. Congr. Cell Biol.* Leiden, 1954 p. 274.
Denny-Brown, D. E. (1929). *Proc. Roy. Soc.* B **104**, 371.
Eastlick, H. L. (1937). *J. Morphol.* **61**, 399.
Edwards, G. A., and Ruska, H. (1955). *Quart. J. Microscop. Sci.* **96**, 151.
Edwards, G. A., Ruska, H., Souza Santos, P. d., and Vallejo-Freire, A. (1956). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 143.
Edwards, G. A., Ruska, H., and de Harven, E. (1958). *J. Biophys. Biochem. Cytol.* **4**, 107.
Fawcett, D. W., and Selby, C. C. (1958). *J. Biophys. Biochem. Cytol.* **4**, 63.
Fenn, W. O. (1945). *In* "Physical Chemistry of Cells and Tissues" (R. Hober, ed.), Sect. 7, Blakiston—Div. McGraw-Hill, New York.
Fernández-Morán, H., and Finean, J. B. (1957). *J. Biophys. Biochem. Cytol.* **3**, 725.
Freeman, J. A. (1956). *J. Biophys. Biochem. Cytol.* **2** *Suppl.*, 353.
Gansler, H. (1956). *Arch. pathol. Anat. u. Physiol. Virchow's* **329**, 235.
Gansler, H. (1957). *Proc. Stockholm Conf. Electron Microscopy* 1956, 190.
Geren, B. B., and Schmitt, F. O. (1954). *Proc. Natl. Acad. Sci. U. S.* **40**, 863.
Geren, B. B., and Schmitt, F. O. (1955). *Symposium 8th Intern. Congr. Cell Biol. Leiden*, 1954 p. 251.
Greene, C. W. (1913). *Bull. U. S. Dept. Fisheries* **33**, 73.
Gutmann, E., and Young, J. Z. (1944). *J. Anat.* **78**, 15.
Haggqvist, G. (1931). *In* "Handbuch der mikroskopischen Anatomie des Menschen" (W. v. Mollendorff, ed.), Vol. 2, (Pt. 3), Springer, Berlin.
Hamoir, G. (1956). *J. Physiol. (London)* **48**, 155.
Hampton, J. C. (1958). *Acta Anat.* **32**, 262.
Harman, J. W. (1955). *Am. J. Phys. Med.* **35**, 68.
Heidenhain, M. (1911). *In* "Handbuch der Anatomie des Menschen" (K. v. Bardeleben, ed.), Vol. 8, Fischer, Jena.
Henle, J. (1841). *In* "Vom Baue des menschlichen Korpers" (S. T. von Sommerring, ed.), Voss, Leipzig.
Huxley, A. F. (1957a). *J. Physiol. (London)* **135**, 17.
Huxley, A. F (1957b). *Progr. in Biophys. and Biophys. Chem.* **7**, 255.
Huxley, A. F , and Niedergerke, R. (1954). *Nature* **173**, 971.
Huxley, A. F., and Taylor, R. E. (1955a). *J. Physiol. (London)* **130**, 49.
Huxley, A. F., and Taylor, R. E. (1955b). *Nature* **176**, 1068.
Jones, W. M., and Barer, R. (1948). *Nature* **161**, 1012.
Kabat, E. A. (1956). "Blood Group Substances: Their Chemistry and Immunochemistry", Academic Press, New York.
Katz, B. (1948). *Proc. Roy. Soc* B **135**, 506
Katz, B (1956). *Brit Med. Bull.* **12**, 210.
Knoll, P. (1891). *Denkschr. Akad Wiss Wien.* **58**, 633.
Kolliker, A. (1857) *Z. wiss. Zool* **8**, 311.
Kolliker, A. (1888). *Z. wiss Zool.* **47**, 689.
Lindner, E. (1957) *Z Zellforsch. u mikroskop. Anat.* **45**, 702.
Low, F. N. (1956a). *J. Appl. Phys.* **27**, 1398.
Low, F. N. (1956b) *Anat. Record* **124**, 462
Luna, E. (1911). *Arch. Zellforsch.* **6**, 383.
Mark, J. S. T. (1956). *Anat. Record* **125**, 473
Moore, D. H., and Ruska, H (1957) *J. Biophys. Biochem. Cytol.* **3**, 261.
Moore, D. H., Ruska, H , and Copenhaver, W. M. (1956). *J. Biophys. Biochem. Cytol.* **2**, 755.
Muir, A R. (1957). *J. Biophys. Biochem. Cytol.* **3**, 193

CHAPTER VII

The Molecular Basis of Contraction in Cross-Striated Muscles

H. E. Huxley and J. Hanson

I. INTRODUCTION

In several fields of biology today, studies which formerly belonged to entirely different disciplines are beginning to find common ground, and it is becoming increasingly possible to formulate many of the more fundamental questions in fairly detailed molecular terms. A number of these questions—for example how chemical energy is stored, released, and converted into other forms, or how enzymes work—are ones which occur in several different contexts, and gains in understanding in one field may help to throw light on others. A cross-striated muscle functions in such a dramatic and efficient manner, and many of its physiological and biochemical properties have been so clearly defined, that it now presents a very favorable system for the study of molecular

far as we know they extend for the whole length of the fiber. The cross striation which is visible in the muscle fiber in the light microscope is due to the striation of its fibrils, which are aligned with equivalent bands in register. Between them are many interesting structures which are described elsewhere in this book and which are concerned in providing sources of chemical energy for the fibrils to use, and in controlling the contractile activity of the fibrils in other ways. It is important to note that the fibrils have no surface membranes (Hodge *et al.*, 1954) (Figs. 5, 6, and 7) so that many of the substances in solution in the cytoplasm ("sarcoplasm") may be present between the protein filaments in the fibrils, and exchange of some metabolites may be relatively unrestricted.

II. THE STRUCTURE OF A FIBRIL

A. Introduction

The fibrils of these muscles, as seen in the light microscope or electron microscope (Figs. 1, 3, and 4), are transversely striped in a very regular manner; the striation arises from a variation of density along the length of the fibril. Each repeat of the pattern is known as a sarcomere. There are considerable differences in sarcomere length between different muscles, and even in one case (blowfly flight muscle—Hanson, 1956) between different fibrils in the same muscle. In general, sarcomeres are usually not more than about 15 μ long; in vertebrate animals they are commonly 2–3 μ long. Exceptionally long sarcomeres (33 μ) have been found in the pharyngeal muscles of certain syllids (annelids) (Haswell, 1889). No correlation between sarcomere length and function has yet been clearly established (Pringle, 1957).

The structure of myofibrils has been analyzed by two complementary methods. Regularly-ordered structures of suitable dimensions diffract X-rays in a manner which gives information about the positions of submicroscopic components. The protein filaments in myofibrils are regularly arranged, and diffraction patterns which show some features of the arrangement of these filaments have been obtained from whole "living" muscles (H. E. Huxley, 1951, 1952, 1953a). This information is necessarily incomplete, but it is valuable because it refers to muscles which are still excitable and contractile. Electron microscopy enables us to examine in more detail the structure of *fixed* muscles. The existence of the filaments and a number of other important features of the structure of myofibrils were revealed by some of the first electron micro-

architecture and function, and their relation both to the large-scale properties of muscle, and to the mechanisms of enzyme action and energy transfer from the chemical to the mechanical form. The studies

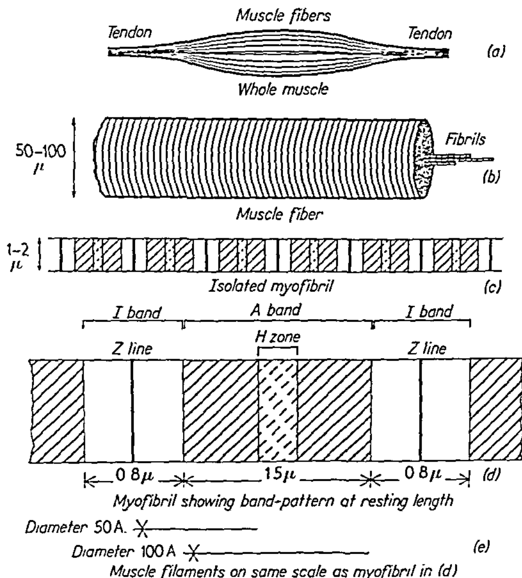


FIG. 1 Diagram illustrating the dimensions and arrangement of the contractile components in a muscle The whole muscle (a) is made up of fibers (b) which contain cross-striated myofibrils (c, d) These are constructed of two kinds of protein filaments (e) put together as shown in Fig 2.

we shall describe in this chapter have these aims, and the results so far show that this approach may be a very fruitful one.

The contractile elements in a cross-striated muscle are the myofibrils inside the fibers (Fig. 1). They are usually 1–3 μ in diameter, and so

far as we know they extend for the whole length of the fiber. The cross striation which is visible in the muscle fiber in the light microscope is due to the striation of its fibrils, which are aligned with equivalent bands in register. Between them are many interesting structures which are described elsewhere in this book and which are concerned in providing sources of chemical energy for the fibrils to use, and in controlling the contractile activity of the fibrils in other ways. It is important to note that the fibrils have no surface membranes (Hodge *et al.*, 1954) (Figs. 5, 6, and 7) so that many of the substances in solution in the cytoplasm ("sarcoplasm") may be present between the protein filaments in the fibrils, and exchange of some metabolites may be relatively unrestricted.

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graphs of fragmented muscles (for example those of Hall *et al.*, 1946; Draper and Hodge, 1949). More recently, the technique of ultrathin sectioning has made it possible to examine fibrils in which the spatial relations between the various components are fairly well preserved (Bennett and Porter, 1953; H. E. Huxley, 1953b, 1957; Hodge *et al.*, 1954; Hodge, 1955, 1956; Spiro, 1956; Huxley and Hanson, 1957b; Sjöstrand and Andersson, 1956, 1957). Some distortion of structure,

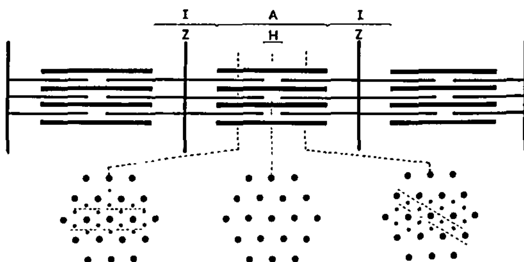


FIG. 2. Diagram illustrating the arrangement of the two kinds of protein filaments in a myofibril. At the top are three sarcomeres drawn as they would appear in [illegible] which is indicated [illegible] transverse section) it would show *two* thin filaments between each two thick ones, as in the electron micrograph in Fig. 5.

and particularly of dimensions, is an inevitable consequence of the methods that must be used in the preparation and examination of these thin sections. However, it is possible to assess some of the artifacts—including those affecting dimensions—by referring not only to X-ray diffraction patterns, but also to the appearance of muscle fibers or myofibrils viewed in a phase contrast or interference microscope, while they are still sufficiently lifelike to be contractile (Huxley and Hanson, 1954, 1957a; Huxley and Niedergerke, 1954; Hanson and Huxley, 1953, 1955; Hanson, 1956; A. F. Huxley, 1957). The description of the fibril which follows is based on the results obtained by using all these

methods. It most probably applies to all cross-striated muscles, although only some vertebrates and arthropods have yet been studied. There is disagreement between various authors in the interpretation of some crucial features of the electron micrographs, but H. E. Huxley (1957) has shown that this can be resolved if one takes account of the effect of the thickness of the sections and if one relies on those which are sufficiently thin to contain only one layer of filaments.

B. The Arrangement of the Filaments

The fibril is constructed (Figs. 2, 5, and 7) from protein filaments which lie parallel to its long axis and are arranged in a series of overlapping arrays. There are two kinds of filaments, and in each array all the filaments are of the same type and extend from one end of the array to the other. The two kinds of filaments differ in diameter, one being about twice as thick as the other, and they also differ in length. The two types of array which they form alternate along the length of the fibril, and where the arrays overlap, the thick and thin filaments interdigitate with each other.

As a result of this construction, a fibril viewed in the light microscope (Figs. 1 and 3) is seen to consist of transverse bands of differing optical density and birefringence. The arrays of thick filaments are the so-called A bands, which are dense and anisotropic. Where the arrays of thin filaments are present alone, the fibril is less dense and less birefringent; these bands are known as the I bands, for they appear to be nearly isotropic (Schmidt, 1934); they are bisected by Z membranes. In either half of the A band, the arrays of thick and thin filaments interdigitate and here the optical density of the fibril is greatest. In the center of the A band, there is a short region where the thick filaments alone are present; this is the H zone, and its optical density is lower than that of the rest of the A band.

The approximate lengths of the two kinds of filaments in living muscles can be measured in the light microscope, for the thick filaments have the length of the A band, and the thin ones extend from one H zone to the next (though it is not clear whether they are actually continuous at the Z lines). In vertebrate skeletal muscles (frog and rabbit), the thick filaments are about 1.5μ long and the thin ones about 2.0μ (or 1.0μ if they are interrupted at the Z lines).

The lengths of A and I bands vary considerably from one kind of muscle to another and, as we shall see later, may control one important



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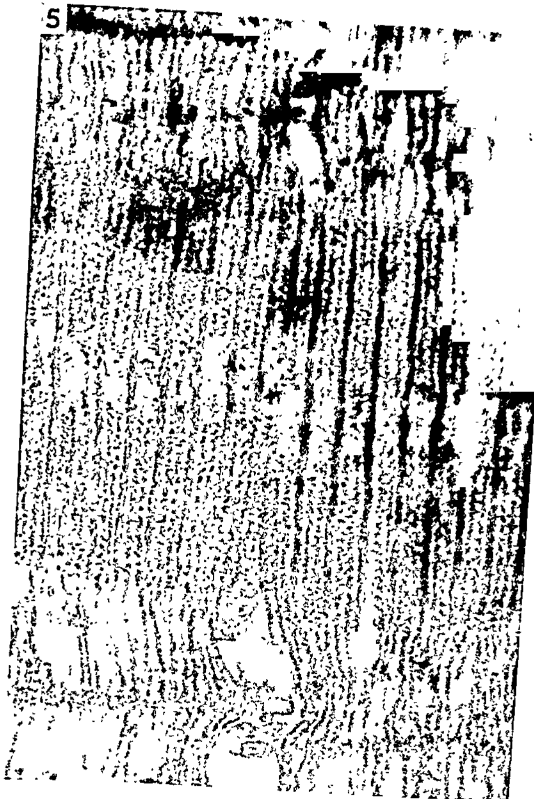
aspect of the behavior of the living muscle. Here we shall only mention that in the indirect flight muscles of *Calliphora*, which shorten very little when they contract, the I bands in resting muscles are so short that they cannot be measured in the light microscope; the A bands are about 3.0 μ long (Hanson, 1956). In the rabbit psoas muscle, held at the maximal length it has in the body, the A band is 1.5 μ long, the I band 0.8 μ (Huxley and Hanson, 1954); this muscle shortens much more than the insect flight muscle when it contracts.

In living vertebrate skeletal muscles, the thick filaments lie 450 A. apart, this being the distance from the center of one filament in the array to the center of the next. Their arrangement is hexagonal. Where the arrays of thick and thin filaments interdigitate, the thin filaments lie in the trigonal positions within the hexagonal array of thick filaments (Figs. 2 and 7). Thus, each thick filament is encircled by six thin ones, and each thin filament is "shared" by three thick ones. In the indirect flight muscles of the blowfly *Calliphora* (Huxley and Hanson, 1957b), the thick filaments are again hexagonally arranged, but the thin ones are relatively more numerous, and each of them is "shared" by *two* thick filaments (Fig. 11).

The diameters of the two kinds of filaments as seen in electron micrographs are about 110 A. and 50 A. It is not known how thick they are in living muscles; the images seen in electron micrographs are of protein filaments which have been dehydrated and heavily stained. The only muscles which have so far been studied in any detail are those of vertebrates and insects. Others may have filaments of different dimensions; very large filaments, about 250 A. in diameter, were isolated from crab leg muscles by Farrant and Mercer (1952).

FIG. 3. A photograph, taken in an interference microscope, of a single glycerol-extracted myofibril from the psoas muscle of a rabbit. The various bands differ in optical density; they are illustrated and named in the diagram (d) in Fig. 1. Such [illegible]

[illegible] as they do in the light micrograph (Fig. 3) and for the same reason; the bands differ in the amount of material they contain. Here the filaments are visible, thick ones in the A band, and thin ones in the I band extending into the A band; they are shown at higher magnification in Fig. 5. Magnification: × 53,000.


5

C. Z and M Lines

Halfway along each array of thin filaments, a thin band of dense amorphous-looking material fills in the spaces between the filaments (Fig. 5); thus the array appears to be bisected by a membrane; this is visible in the light microscope and is known as the Z line or Z membrane. The filaments are slightly thickened in the vicinity of the Z line.

The thick filaments taper at either end and are slightly thickened just in the middle (Figs. 4, 5, and 10); as a result, the A band appears to be bisected by an ill-defined line. This is the position of the so-called M line which has been observed in light microscope images, particularly of insect fibrils; but, as we shall see, a different kind of dense line (involving the thin filaments) appears in this position in contracted fibrils, and it is not always clear which type of line is referred to in the literature on this subject. In addition to the Z and M lines, other transverse lines have been seen in both the I bands and the A bands of insect and vertebrate myofibrils, but they have not yet been studied in any detail.

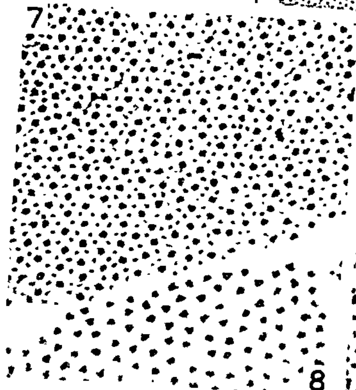
D. Bridges between Filaments

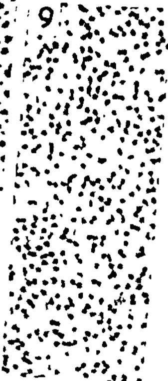
Each of the thick filaments in a cross-striated muscle bears a large number of regularly spaced short lateral projections, which always appear to lie more or less at right angles to the filament axis. They extend towards and appear to touch the thin filaments, where these are present in the array of thick filaments (Fig. 10). In the psoas muscle of the rabbit, where their arrangement has been studied in some detail (H. E. Huxley, 1957), there are six longitudinal rows of these projections on each thick filament, and in each row there is one projection

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Fig. 5. An electron micrograph of a longitudinal section through the sarcomeres of two adjacent myofibrils, such as those shown at lower magnification in Fig. 4. The Z lines bounding the sarcomeres are at the top and bottom of the picture. Two kinds of filaments are visible, thick ones (about 110 A.) in an array confined to the A band, and thin ones (about 50 A.) in two arrays which terminate at the borders of the H zone in the middle of the picture. The two kinds of filaments interdigitate in the A band (except the H zone), the plane of sectioning through the lattice of interdigitating filaments which will produce a longitudinal section like this one is illustrated in Fig. 2 (p. 186). Compare Fig. 21, cut in a different plane. Cross links between thick and thin filaments are visible; these are shown at higher magnification in Fig. 10. Magnification × 147,000.


6


7
8


9

every 400 A., approximately.[1] The projections in the six rows are "staggered," so that every 60-70 A. along the thick filament, there is one projection. The rows are arranged so that they occur opposite the six thin filaments which lie alongside. (It will be recalled that in the A band, where the arrays of thick and thin filaments interdigitate, each thick filament is encircled by six thin ones, and each thin filament is "shared" by three thick ones.) Hence, from the three thick filaments which lie equidistant from each thin one, a lateral projection reaches out to this thin filament every 130-140 A. along its length.

The methods for preparing very thin sections for electron microscopy have only been developed in the last few years. Previously, studies were made on whole isolated myofibrils or on rather thicker sections. These showed an axial periodicity (Fig. 17), usually of about 400 A., but sometimes much less (see for example, Hall *et al.*, 1946; Draper and Hodge, 1949; Hoffmann-Berling and Kausche, 1950; Hodge, 1956). This axial periodicity is visible not only in the A bands but also in the I bands, whose filaments do not bear lateral projections. There are several possible interpretations, some of which are discussed later (p. 203-206). Similarly, it is not yet clear what produces the axial periodicity of 415 A. revealed in "living" muscles by low-angle X-ray diffraction methods (H. E. Huxley, 1951, 1952, 1953a). Since the different proteins in these myofibrils must be fitted together in a very precise manner, any regularities the composite structure possesses may be due to the molecular structure of more than one of its constituents.

[1] The distance from one projection to the next along a filament has been estimated by counting the *number* of projections in electron micrographs, and dividing this into the *length* of the filament, as measured from the band lengths of living muscles in the light microscope.

FIG. 6. An electron micrograph of a transverse section through several myofibrils in a rabbit muscle such as Figs. 4 and 5 show in longitudinal section. The section has [illegible] digitating arrays [illegible] × 44,000.

[illegible] filaments (about 110 A.) are hexagonally arranged. The thin ones (about 50 A.) are situated in the trigonal positions in this lattice; thus each thick filament is encircled by six thin ones, and each thin filaments is "shared" by three thick ones (see diagram in Fig. 2, p. 186). Magnification. × 132,000.

FIG. 8 A transverse section passing through the H zone where only the thicker filaments are present. Magnification. × 132,000.

FIG. 9. A transverse section passing through the I band where only the thin filaments are present, and their arrangement has been disturbed. Magnification: × 132,000.

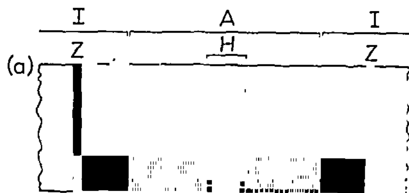

10
11
100A

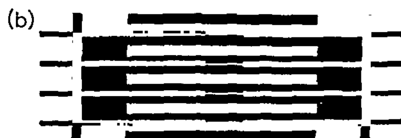
E. Relative Amounts of "A Substance" and "I Substance"

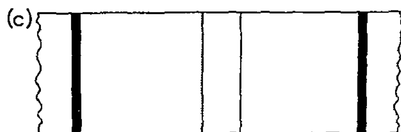
An interference microscope can be used to estimate the quantity of material other than water in living cells and similar objects (Davies *et al.*, 1954). In order to obtain absolute values, the dimensions must be known. The thickness of a myofibril cannot be measured accurately. However, it has been possible to determine the *relative* quantities of materials in the different parts of the glycerol extracted rabbit psoas sarcomere (Huxley and Hanson, 1957a); these materials are practically all proteins (Perry, 1955). The methods used for this analysis cannot be critically discussed with any brevity; here we shall have to summarize the results without describing how they were obtained (Figs. 13 and 14). "A substance" is defined as the optically dense material which makes the A band denser than the I band; it is believed to correspond to the material of the thick filaments. "I substance" corresponds to the material of the thin filaments; it extends from one H zone to the next, so that for some part of its length it is situated in the A band. These two "substances" can be experimentally distinguished, because one of them, the A substance, can be extracted completely from the fibril, leaving most of the I substance behind. Of the total dry mass of the sarcomere, about 54% is A substance, 36% is I substance, and 6% is Z line material. A small quantity of other material bridges the H zone where the I substance is discontinuous in the middle of the A band. The A band (apart from the H zone) is three times as dense as the I band, i.e. the A substance is twice as dense as the I substance. As there are twice as many thin filaments in the I substance as there are thick filaments in the A substance, it follows that unit length of a thick filament must contain four times as much material as unit length of a thin filament; this is consistent with the observed filament diameters, providing their densities are similar.

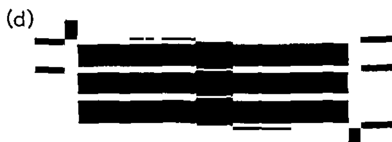
Fig. 10. An electron micrograph at high magnification of a longitudinal section through part of a rabbit fibril including the H zone and the adjacent parts of the A band. A similar section at a lower magnification is shown in Fig. 5. The picture here shows the cross links between the thick and thin filaments. The impression that some links connect the thin filaments to each other is probably due to the visibility of links belonging to thick filaments which lay above and below the plane of this section. Magnification × 510,000.

Fig. 11. An electron micrograph of part of a transverse section through the A band of a myofibril in the indirect flight muscles of the fly *Calliphora*. Here, as compared with rabbit muscle (Fig. 7), the thin filaments lie in different positions with respect to the thick ones, and the cross links are more clearly visible. Magnification: × 170,000.


I
A
I
Z
H
Z
(a)


(b)


(c)


(d)

F. Summary

A fibril in a cross-striated muscle is constructed of an alternating series of arrays of longitudinal filaments of two kinds. Their sequence results in the regular alternation of A and I bands, which is the characteristic microscopical feature of this type of muscle. The arrays overlap and their filaments interdigitate in the A band; here a large number of projections from the thicker filaments reach towards and appear to touch the thinner filaments.

III. THE PROTEINS OF THE FILAMENTS

Rabbit skeletal muscles have been employed much more than any others for biochemical studies and, as we have seen, their structure is known in some detail. Thus it has been possible to devise experiments to discover the location of the chief proteins in the contractile elements of rabbit muscles. The results probably apply to other cross-striated muscles as well, for it is known that they also possess the three proteins which account for nearly all the dry matter of the rabbit fibril—myosin, actin, and tropomyosin.

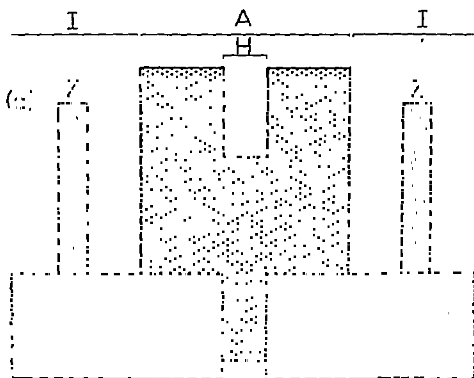
Myosin is quantitatively the most important. It accounts for about 54% of the total protein in the fibrils (Hanson and Huxley, 1957; Huxley and Hanson, 1957a). Tropomyosin is present in smaller amounts, about 11% (Perry and Corsi, 1958). The quantity of actin is less well known; a reasonable estimate is 20–25% (Hanson and Huxley, 1957; Perry and Corsi, 1958). It is unlikely that any other proteins are present in large amounts. Indeed, it is possible that the figures we give for actin and tropomyosin are too low; the fraction which is unaccounted for (10%) is not necessarily another material.

A. Myosin

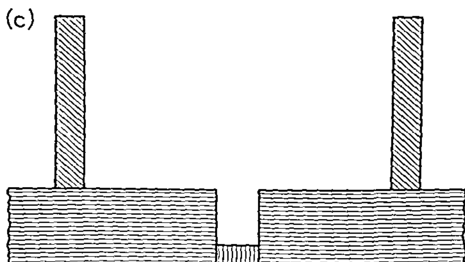
Myosin can be extracted from these muscles (see also Volume II, Chapter I) by hypertonic salt solutions. When pyrophosphate and

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Fig. 12. Diagrams illustrating the structural effects of the extraction of myosin from a sarcomere· (a) the bands in an intact sarcomere; (b) the filaments in an intact sarcomere; (c) the bands in an extracted sarcomere; (d) the bands in an extracted sarcomere; (d) the filaments in an extracted sarcomere. As a result of extraction, the A substance (the thick filaments) disappears, leaving behind the I substance (thin filaments) and Z lines. Photographs which show this effect are illustrated in Fig. 14.



(b)



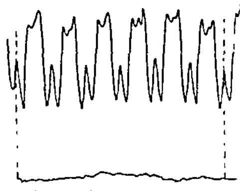
magnesium ions are also present in these salt solutions, the extraction of actin is largely suppressed (Hasselbach and Schneider, 1951; Hasselbach, 1953). The reason seems to be that pyrophosphate, in the presence of magnesium, dissociates the complex of actin and myosin (actomyosin) which exists in the fibrils, and that actin is not readily extracted except in combination with myosin. It is not known exactly how much tropomyosin comes out with the myosin. However, tropomyosin, once it has been extracted from the muscle, is soluble in weak salt solutions, and actin is also soluble, whereas myosin is insoluble. Therefore the amount of myosin in the extracted protein solution can be estimated by reducing the ionic strength of the extract and measuring the quantity of protein that precipitates; such estimates are not completely accurate, for a variable but small part of the myosin fails to precipitate.

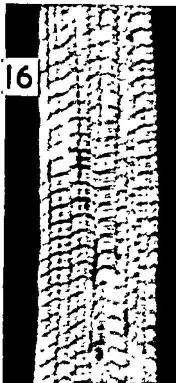
When this method for the extraction and estimation of myosin was applied to isolated rabbit myofibrils (Hanson and Huxley, 1957), it was found that about 51% of their total protein content is myosin. A very similar value can be deduced from the results of Hasselbach and Schneider (1951) obtained for whole muscle. Szent-Györgyi *et al.* (1955) and Perry and Corsi (1958) also obtained rather similar figures. This quantity (51%) is nearly the same as that of the A substance in these fibrils, strongly suggesting that myosin is the A substance.

The same extraction method has been applied to similar fibrils under the light microscope (Hanson and Huxley, 1953, 1955; Huxley and Hanson, 1957a), and the residue after extraction has also been studied in the electron microscope (Hasselbach, 1953; Hanson and Huxley, 1953, 1955). In a phase contrast or interference microscope, it is immediately seen that the A substance disappears (Figs. 12, 14, and 15). The I substance and the Z lines persist, and seem to be unchanged, and the extracted sarcomere consists of material of uniform optical density which is, however, interrupted in the H zone; here there appears to be a gap, but this must in fact contain some small amount of material, for the fibrils do not fall apart into segments, and indeed it has been possible to detect and measure the material in the gap by interference

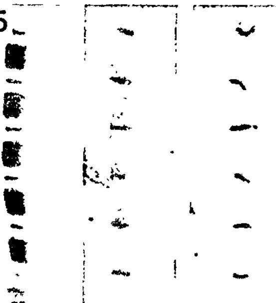
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FIG. 13. Histograms representing quantitatively the distribution of protein in a single sarcomere: (a) intact sarcomere; (b) the A substance; (c) sarcomere after extraction of myosin (A substance). Vertical dimensions represent the proportion of each component in the total protein. The results on which these histograms are based were obtained by interference microscopy (see Fig. 14).


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microscopy. When the process of extraction is viewed in a polarizing microscope, it is seen that the birefringent A bands disappear. Electron micrographs of extracted fibrils not only confirm that the A substance has been removed, but also show that it is the *thick* filaments of the A band which have disappeared.

Interference microscopy has been used to estimate the amounts of material that are removed from the different parts of the sarcomere (Huxley and Hanson, 1957a) (Figs. 13 and 14). All of the A substance is taken out (i.e. about 54% of the total protein), together with about one-fourth of the I substance, so that, in all, about 60–65% of the total material of the sarcomere is extracted. Parallel estimates by chemical methods (Hanson and Huxley, 1957) showed that on the average 62% of the protein of the fibril is extracted and that most of the extract (51% of the total fibril protein) is myosin.

These results provide very strong evidence that myosin is the A substance, and hence that the thick filaments of the fibril are composed of myosin.

There is another way of approaching the problem of the location of proteins in myofibrils. Finck *et al.* (1956) and Holtzer *et al.* (1957) have

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Fig. 14. Photographs taken in an interference microscope (and densitometer tracings of the negatives) of one glycerol-extracted rabbit fibril before and after the extraction of myosin. The A substance is removed, leaving behind the I substance and the Z lines. To the eye, there appear to be gaps, where the I substance is discontinuous, in the center of the sarcomere (H zone), but the densitometer tracing demonstrates that there is a small amount of material here. The tracings were taken along the length of the fibril and show peaks corresponding to Z lines and A bands, and troughs in the I bands. The heights of such peaks or troughs above the base line (tracing of background density) are related to the amount of protein present. Diagrams illustrating the results of such interference microscope studies are shown in Fig. 13. Magnification: × 2,600. (From *Biochim. et Biophys. Acta.*)

Fig. 15. Three photographs, taken in a phase contrast microscope, of a single glycerol-extracted rabbit psoas fibril treated first to extract myosin, and then to extract actin. The A substance disappears on myosin extraction, the I substance on actin extraction. The Z lines persist. Magnification: × 3,350. (From *Symposia Soc. Exptl. Biol.*)

Fig. 16. A photograph taken in a fluorescenc
chick muscle treated with a fluorescein-labeled
The A bands are labeled. (Reproduced from Fi
of the authors of this paper and of the editors of the *J. Biophys.* and *Biochem. Cytol.*) Magnification: × 1,300.

Fig. 17. An electron micrograph of a longitudinal section through rabbit muscle showing the small-scale axial periodicity in A and I bands. Magnification: × 33,000.

found that a fluorescently-labeled antibody to myosin will react with the A bands rather than with the I bands of chick muscles (Fig. 16).

Extraction experiments have also been performed on insect flight muscles, and have given qualitatively similar results (Hanson, 1956). There is also some similar evidence that the A substance in the arachnid, *Limulus*, is myosin (Amberson *et al.*, 1949).

B. Actin

If the thick filaments are composed of myosin, then actin must be situated in the thin filaments, which make up the I substance. Qualitative evidence that this is so has been provided by extraction experiments of two kinds. A. G. Szent-Györgyi (1951a, b) showed that actin can be extracted from rabbit muscles by potassium iodide, which depolymerizes the fibrous aggregates of actin molecules in the fibrils. When a potassium iodide solution was applied to myosin-free fibrils under the microscope, it was found that a large part of the I substance was extracted (Hanson and Huxley, 1955) (Fig. 15). More recently, Perry and Corsi (1958) have found that a great deal of protein, sometimes as much as one-third of the total, slowly leaches out of isolated fibrils stored in solutions of low ionic strength. The extracted protein consists of tropomyosin (identified electrophoretically) and of another protein which closely resembles a form of actin that can be produced from "normal" actin when this is submitted to the same procedures as were used for extracting the fibrils. Corsi and Perry (1958) examined these fibrils in a phase contrast microscope and concluded from the changes observed as a result of extraction that tropomyosin and actin were situated mainly in that part of the sarcomere which is here called "I substance." They found that this type of extraction, which sometimes removed the I bands and Z lines completely, appeared to leave all the myosin behind in the residue.

A different kind of experiment (Hanson and Huxley, 1955) again indicates that the I substance contains actin. It is well known that actin and myosin *in vitro* form a complex—actomyosin—which contracts when it is treated with ATP; neither protein by itself is contractile (Hyashi *et al.*, 1958). Rabbit fibrils from which the A substance had been removed were treated with a solution of rabbit myosin under the microscope. The protein was taken up by the I substance, whose optical density increased. When these "reconstituted" fibrils were treated with ATP, the I substance in each half of the sarcomere contracted to the

adjacent Z line where a thick "contraction band" was formed. If ATP was given *before* myosin, it had no effect on the fibril. Similar results were obtained (Hanson, 1956) when *insect* fibrils, freed from A substance, were treated with *rabbit* myosin and then with ATP. It is clear that in these experiments, an actomyosin system had been formed from the thin filaments and the added myosin.

C. Summary

There is strong evidence that the thick filaments are composed of myosin, and that the thin filaments contain actin and perhaps tropomyosin.

IV. THE MOLECULAR STRUCTURE OF THE FILAMENTS

Information from several sources provides evidence about the molecular structure of the filaments. In the first place, one can study the molecular weights and dimensions of the principal muscle proteins in solution. Next, one can prepare oriented arrays of these protein molecules, which give X-ray diffraction patterns, and sometimes show axial periodicities in the electron microscope. Now, X-ray patterns are essentially of two types: "low-angle" patterns produced by large-scale regularities in the structure (up to about 0.1 μ), and "wide-angle" patterns which derive from small-scale regularities of only a few Angström units. Studies of these patterns can therefore give us information both about the way in which the protein molecules can pack together, and about the nature and arrangement of the polypeptide chains within these molecules. Finally, we can make similar studies on the structure of the filaments as they occur in the muscle, and use the information obtained from the simpler artificial systems to assist in the interpretation of the results.

A. The Myosin Filaments

The number of molecules in a single myosin filament can be estimated approximately in the following way. 100 g. of rabbit muscle contains 20 g. of dry matter, of which 7.6 g. is myosin (Hasselbach and Schneider, 1951). The number of myosin filaments in a myofibril of given length and cross section can be calculated from the data already described. A very high proportion of the muscle volume is occupied by fibrils, but the exact figure is not known. Here we shall assume, for the sake of simplicity, that the muscle consists entirely of fibrils. If

this were so, then the "molecular weight" of one myosin filament would be 180 millions. The molecular weight of myosin is difficult to measure accurately, partly because the molecules in solution tend to aggregate. Recent estimates suggest a weight of about half a million (Laki and Carroll, 1955; Holtzer and Lowey, 1956). A value which is probably more reliable can be deduced from studies on the "light" and "heavy" meromyosins into which myosin splits when it is briefly treated with trypsin (A. G. Szent-Györgyi, 1953). The meromyosins are liberated in the proportion of one H-meromyosin to two L-meromyosins, and their molecular weights are 236,000 and 94,000 respectively. Hence the molecular weight of myosin itself should be 424,000 or some multiple of this. If the molecular weight of myosin is taken to be 424,000, the number of molecules in each filament is about 424.

There is evidence that the myosin molecules lie with many of their polypeptide chains lengthwise in the muscle, and that these chains have an α-helical configuration (see also Volume II, Chapter I). The wide-angle X-ray diffraction pattern of whole muscle is that of a structure containing longitudinally-oriented α-helical polypeptide chains (Pauling *et al.*, 1951; Pauling and Corey, 1951; Perutz, 1951; Huxley and Perutz, 1951). A very similar pattern is given by oriented samples of actomyosin (Astbury and Dickinson, 1940), but actin itself has a wide-angle pattern of a different type (Astbury and Spark, 1947; Cohen and Hanson, 1956). Tropomyosin gives a pattern like that of whole muscle (Astbury *et al.*, 1948) but is present in much smaller amounts than myosin.

The "staggered" arrangement of the lateral projections on the myosin filaments suggests that the molecules may also be "staggered." The observation that the filaments taper at their ends supports this suggestion. Numerically, there seem to be two myosin molecules for each lateral projection. The number of projections counted in electron micrographs is about 216 on each filament, while the calculated number of molecules is 424.

The meromyosins to which we have briefly referred seem to exist as definite units in the myosin molecule. When they are released, they always have the same dimensions and properties, and they can also be prepared from myosin by using chymotrypsin (Gergely *et al.*, 1955), which differs from trypsin in normally attacking different links in polypeptide chains; subtilisin also releases meromyosins (Middlebrook, 1958). There is some evidence that they are synthesized independently

of each other in the body (Velick, 1956). The wide-angle X-ray diffraction pattern of an oriented sample of L-meromyosin (Cohen and A. G. Szent-Györgyi, personal communication) is very similar and rather superior to that of whole muscle. Repeating the argument we used to show that myosin molecules probably lie lengthwise in the filaments, we now deduce that the L-meromyosin units may be similarly oriented.

Light meromyosin can precipitate from solution as transversely-striped "crystals" with an axial period of 424 A. as measured in the electron microscope (Philpott and Szent-Györgyi, 1954). One is reminded that an axial periodicity of about 400 A. is visible in myofibrils, and that a spacing of 415 A. can be demonstrated in whole "living" muscles by low-angle X-ray diffraction methods. The spacing of the lateral projections in each of the six rows along a myosin filament is also about 400 A. We have already indicated that these projections link the actin and myosin filaments together, and later in this chapter it will become even more apparent that the actomyosin complex, as it exists in muscle, is most probably made by means of these cross-links. Light meromyosin, however, does *not* combine with actin *in vitro*, but heavy meromyosin does, and the actin-H-meromyosin complex is, like actomyosin, dissociated by ATP (A. G. Szent-Györgyi, 1953). This strongly suggests that the lateral projections on the myosin filaments are composed of H-meromyosin. One can imagine that the "backbone" of a myosin filament is composed of longitudinally-oriented "staggered" L-meromyosin units, whose length determines the axial periodicity in these filaments and the positions of the H-meromyosin units; and that the H-meromyosins, attached to the L-meromyosins, project from the filaments and are visible in the electron microscope.

B. The Actin Filaments

The approximate number of actin molecules in each filament can be calculated by the same method as we used for myosin filaments. The amount of actin in 100 g. of rabbit muscle is taken to be 3.5 g. (see p. 197) and the molecular weight, 70,000 (Tsao, 1953a). Each filament (length 2 μ) contains about 600 molecules.

It is very probable that these filaments also contain tropomyosin (Perry and Corsi, 1958; Corsi and Perry, 1958), in the proportion of about 1.7 molecules of actin to 1 of tropomyosin. (The molecular weight of tropomyosin is about 53,000—Tsao *et al.*, 1951.) The relative

positions of these two proteins in the filaments are unknown, but it seems likely that the actin molecules are exposed at the surface to form actomyosin links with the adjacent myosin filaments.

The low-angle X-ray diffraction pattern of an oriented sample of actin (dry or rewetted fiber) (Astbury and Spark, 1947; Cohen and Hanson, 1956) is remarkably like that of whole *dry* muscle (Bear, 1945; Selby and Bear, 1956). (The pattern given by *fresh* muscle is rather different; it shows a strong 415 A. axial periodicity in addition to the 54 A. periodicity characteristic of dry muscle or dry actin.) Samples of myosin or tropomyosin have not so far given any low-angle patterns. For this and other reasons, Selby and Bear have identified the pattern they obtained from the fast part of the clam (*Venus*) adductor muscle as that of actin; this is a smooth muscle (Hanson and Lowy, unpublished) but its X-ray pattern is similar to that of cross-striated muscles (Bear, 1945). Selby and Bear point out that their data could be interpreted in terms either of a two-dimensional net, or of a two-chain helix with a 406 A. axial period and with a diameter comparable to that of the thin filaments in electron micrographs. It is perhaps significant that an axial periodicity of about 400 A. is visible in the I bands of myofibrils where actin filaments but no myosin filaments are present. Fibrous actin *in vitro*, however, shows a 300 A. axial periodicity in the electron microscope (Rosza *et al*, 1949). H. E. Huxley (1957) has pointed out that a two-chain helical model constructed from the data of Selby and Bear would have a threefold axis of symmetry when viewed end-on, and would therefore be very suitable for forming cross-links with three myosin filaments placed symmetrically about it.

Tropomyosin preparations were examined in the electron microscope in 1948 by Astbury *et al.*; there were slight indications of an axial period, and it is important that this protein should be studied again. A different kind of tropomyosin, from molluscan smooth muscles, shows clear axial periodicity, which in some "crystals" is 145 A. and in others, 720 A. (Hanson *et al.*, 1957). There is good reason to believe that this particular tropomyosin is responsible for the low-angle patterns of some of these molluscan muscles (Bear, 1944; Bear and Selby, 1956) where it is present in very large amounts (Bailey, 1956).

It is clear that further X-ray diffraction studies on muscles and their proteins will eventually give us a much more detailed picture of the construction of the filaments in the living muscle.

V. STRUCTURAL CHANGES DURING CONTRACTION AND EXTENSION

There are as yet no methods for observing structural changes at the molecular level while a muscle is contracting, although it is possible that improvements in X-ray diffraction methods will eventually make this feasible. In the meantime, several important things can be learned by studying under the light microscope the large scale changes in contracting muscles. It is also instructive to examine how the structure has been changed *after* the muscle has shortened or been extended to a new length, and this can be done by X-ray diffraction and electron microscopical methods.

A. Results Obtained by Light Microscopy

Several studies have been made on muscles contracting under the microscope; these are reviewed by A. F. Huxley (1957). Two recent investigations have given consistent and unambiguous results and are more reliable than previous ones, because optical artifacts were avoided, in the one case by the use of an interference microscope (Huxley and Niedergerke, 1954; Huxley and Taylor, 1955; A. F. Huxley, 1957) and in the other case by working with single myofibrils which are thin enough for the cross striations to be observed and measured in a phase contrast microscope of high resolving power (Huxley and Hanson, 1954; Hanson and Huxley, 1955; Hanson, 1956). The studies of A. F. Huxley and his colleagues were made on intact frog *fibers* during stretch or isotonic or isometric contraction. Those of Hanson and Huxley were made on glycerol-extracted rabbit psoas *fibrils* (and also on insect flight fibrils) treated with adenosine triphosphate (ATP) either while they were held at constant length or while they were free to shorten; such fibrils could also be stretched if dephosphorylation of ATP was suppressed (see p. 215-216). Other chapters in this book review the convincing evidence that the changes taking place in glycerol-extracted fibrils during ATP-induced contraction, and during stretch, are similar to those occurring in life.

It has been found that the A bands remain at constant length while the length of the fibril is changing. While the fibril shortens, the I bands shorten, while it is being stretched, they lengthen.

The constancy of A band length is observed in rabbit myofibrils over the range from maximal extension down to about 65% of "resting" length[2] (Fig. 19). It must be remembered, of course, that the resolution

[2] "Resting" length is defined as the maximal length of the muscle in the body.

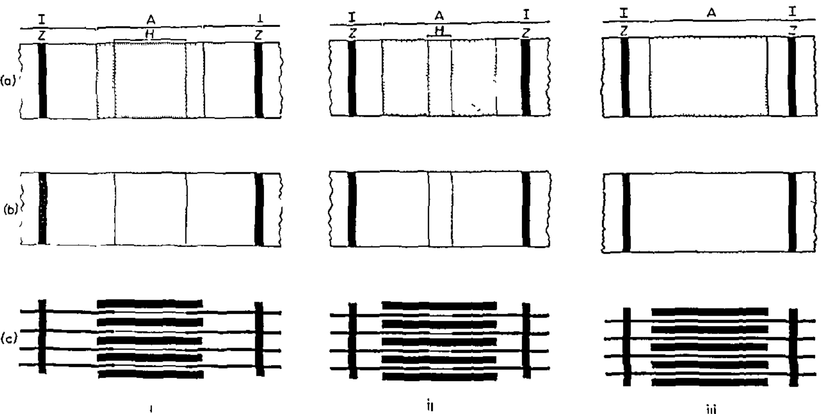


Fig. 18. Diagram illustrating the structural changes associated with contraction (iii) and extension (i) from resting length (ii). The top row (a) shows the band patterns of intact fibrils. The next row (b) shows the band patterns after extraction of myosin. The bottom row (c) shows the positions of the filaments. Light and electron micrographs on which these diagrams are based are shown in Figs. 19 and 22–28.

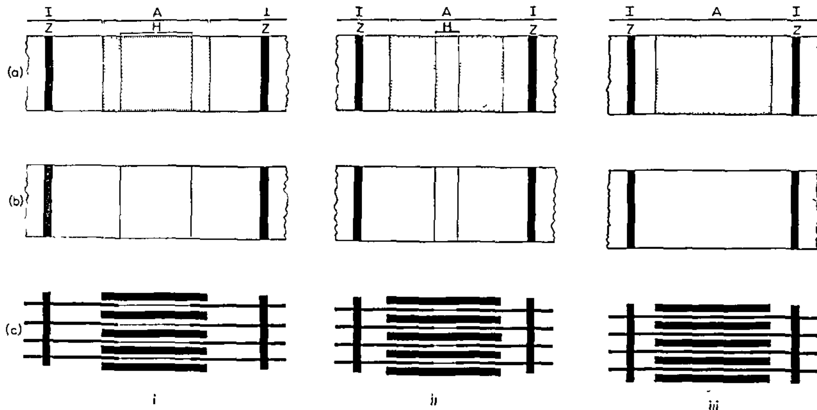


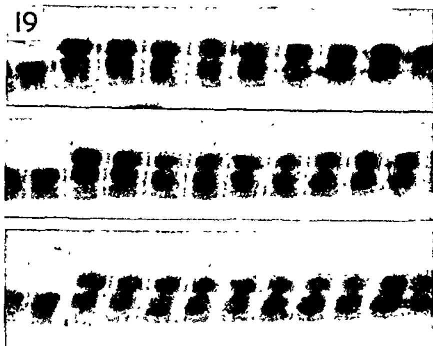
FIG. 18. Diagram illustrating the structural changes associated with contraction (iii) and extension (i) from resting length (ii). The top row (a) shows the band patterns of intact fibrils. The next row (b) shows the band patterns after extraction of myosin. The bottom row (c) shows the positions of the filaments. Light and electron micrographs on which these diagrams are based are shown in Figs. 19 and 22–28.

of the light microscope is limited, and small changes will not be detected. The frog fibers were observed in a microscope of lower resolution, and this may account for the decrease in A band length which was found when the fibers had shortened below about 80% of resting length.

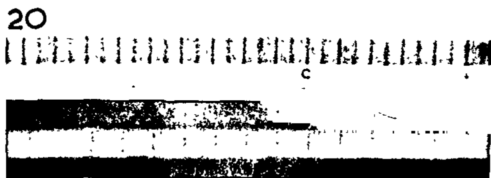
When the rabbit psoas fibril has shortened to about 65% of resting length, the I bands have completely disappeared, and each Z line now touches the ends of the two adjacent A bands. Here, as shortening continues, a dense "contraction band" develops. This becomes the most conspicuous band in the fibril, and a "reversal of striation," such as many earlier workers described, appears to have taken place (Figs. 20 and 22). Probably contraction bands form by distortion of the ends of the myosin filaments, when they are pressed against the Z lines. In polarized light, the A bands remain birefringent, and the contraction bands are isotropic (Fig. 20).

During isometric contraction, neither the A band nor the I band changes its length.

Thus it is clear that the myosin filaments, whose length determines the length of the A band, do not shorten or lengthen by any important amount over the normal working range of the muscle. A further series of observations suggests that the actin filaments also stay at constant length during extension of the muscle and during contraction down to about 90% of resting length (Huxley and Hanson, 1954; Hanson and Huxley, 1955; Hanson, 1956). Thus it is found that the H zone in the middle of the A band widens as the sarcomere is stretched, and closes up as it shortens. In fibrils stabilized at different lengths (by glycerol extraction), one can easily determine the positions and lengths of the actin filaments by extracting the A substance and observing the I substance in the extracted fibril (Figs. 18 and 22). The I substance in a fibril at resting length is about 2 μ long and extends from one H zone to the next; the H zone, after removal of the A substance, looks like a short gap, about 0.25 μ wide. In fibrils which have been stretched, the I substance is still about 2 μ long, but the H zone has become wider, by an extent that depends on how much the muscle has been stretched. Below resting length, the I substance shows no changes in length until the H zone has disappeared, and then it shortens, but a dense line appears in the middle of the sarcomere as though the actin filaments were crumpling at their ends. (This line is visible in intact fibrils, and it is probably the M line described by some previous workers.)


19


21


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C

Thus, over a wide range of lengths, a muscle can contract without any detectable changes taking place in the overall lengths of any of its filaments. And even on further shortening, neither type of filament changes its length until it is obliged to do so for steric reasons occurring as a *result* of shortening.

The results of studies in the light microscope clearly indicate that shortening is brought about by a *sliding* movement of the arrays of actin filaments, inwards into the arrays of myosin filaments in the A bands, and that extension of the muscle reverses this movement.

B. Submicroscopic Changes

Neither the wide-angle nor the low-angle X-ray diffraction pattern of a muscle seems to change in the meridional (axial) direction as a result of extension or contraction (Astbury, 1947; H. E. Huxley, 1951, 1952, 1953a). Many of these studies were made on "living" or glycerol-extracted muscles, and they provide strong evidence that the filaments do not change their lengths as a result of changes in muscle length; for one would expect a shortening or lengthening of a filament to result in alterations in its internal structure and hence in the X-ray patterns. None of the investigations we have so far discussed excludes the possibility that small-scale changes occur *during* contraction.

Although the electron microscope has a high resolving power, it does not provide a straightforward method for measuring filament lengths or band lengths, because these may change while the fibrils are being prepared for examination. However, if suitable precautions are taken, it is possible to *compare* muscles of different lengths with some confidence

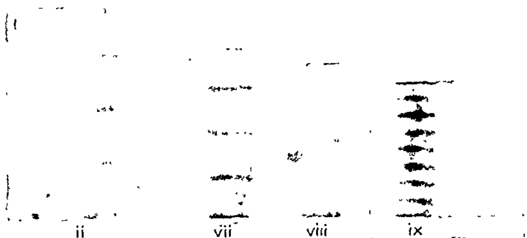
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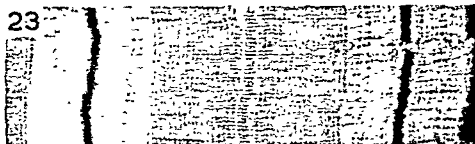
Fig 19. Photographs, taken in a phase contrast microscope, of a pair of glycerol-extracted rabbit myofibrils at different stages during contraction (in ATP), from resting length down to about 80% of that length. It can be seen that the A band stays at the same length while the I bands shorten and the H zone closes up and becomes replaced by a dense line. Magnification: × 4,000.

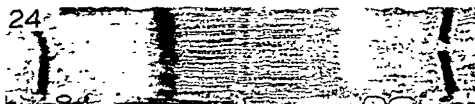
Fig. 20. A contracted fibril isolated from fresh *Drosophila* muscle (indirect flight muscles) and photographed in phase contrast and in polarized light. The I bands have disappeared and contraction bands (c) (isotropic) have developed at the Z lines. A rabbit fibril with contraction bands is shown in Fig. 22, vi (Plate 7). Magnification: × 2,500.

Fig. 21. An electron micrograph of a longitudinal section through one sarcomere of a rabbit fibril (at about resting length). The plane of sectioning is indicated in Fig. 2 (p. 186), and this picture should be compared with that in Fig. 5, which is of a longitudinal section cut in a different plane. Magnification: × 90,000.


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viii
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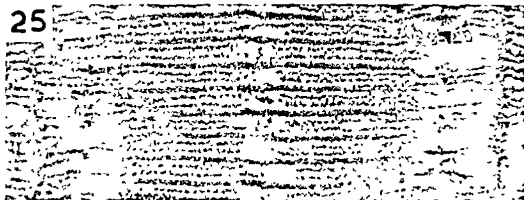
that the results are significant. In this way, it is again found that the length of the A band remains constant and the length of the I band changes with changing muscle length (H. E. Huxley, 1957; H. E. Huxley and B. Katz, unpublished). Moreover, it can be seen that, in contracted muscles, the actin filaments have reached the middle of the A band (Fig. 28); in stretched muscles, the zone of overlap between the arrays of actin and myosin filaments is shorter than it is in resting muscles, and there is a long H zone from which the actin filaments have been withdrawn (H. E. Huxley, 1957). In these stretched muscles, the lateral projections on the myosin filaments are still visible in the zone from which the actin filaments have been withdrawn. At all muscle lengths, the lateral projections are seen to lie more or less at right angles to the filament axis.

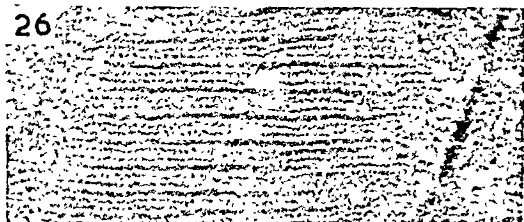
VI. THE MECHANISM OF CONTRACTION

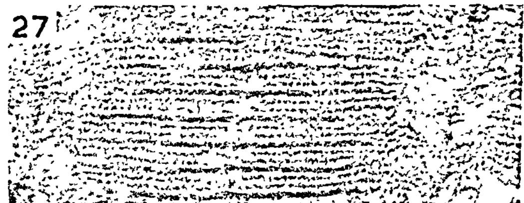
All the information we have discussed leads to the conclusion that contraction is brought about by a mechanism which causes the actin filaments to slide past the myosin filaments in the direction of the center of the sarcomere. We have no direct information about the exact nature of this mechanism, but many of the well-known biochemical and physiological properties of muscles can tell us a great deal about its general properties.

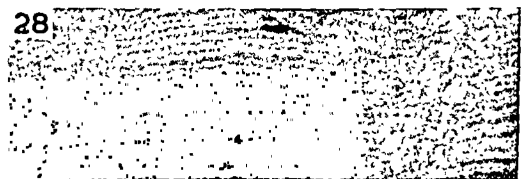
FIG. 22. Photographs showing the changes in band pattern which occur when a fibril contracts or is extended. The top row shows intact fibrils, the bottom row fibrils from which the A substance has been extracted. The latter illustrates how the I substance changes position during contraction and extension. Compare with Fig. 18 (p. 208). (i) and (ii): a stretched fibril (110% resting length) before and after myosin extraction; there is a very wide H zone, which shows as a wide "gap" after extraction. (iii)–(vi): one fibril photographed at different stages during contraction, in ATP, from resting length (iii) down to about 50% of that length (vi). The H zone closes up (iv) and is replaced by a dense line (v); when the I bands have disappeared, contraction bands form at the Z lines (vi). (vii)–(ix): three fibrils, similar in length to those above, from which myosin has been extracted. In (viii), the H zone ("gap") has disappeared and in (ix), a dense line has appeared in the center of the sarcomere. Magnification: × 3,900.

FIGS. 23 and 24. Electron micrographs of longitudinal sections through glycerol-extracted rabbit fibrils, one (Fig. 23) at about resting length, the other (Fig. 24) stretched by about 30%. The A bands have the same lengths, but extension has elongated the I bands and the H zones. Magnification: × 28,000.


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A. Actomyosin Systems

When ATP is added to a solution of actomyosin, a decrease in viscosity and turbidity is observed, and this is believed to be due to the dissociation of actomyosin into actin and myosin (Straub, 1942, 1943; A. Weber, 1956; Gergely, 1956). For as long as enough ATP is present, the proteins remain dissociated, but when the ATP concentration diminishes—by dephosphorylation, catalyzed by myosin—actomyosin reforms.

Actomyosin precipitated from solution contracts when it is treated with ATP (A. Szent-Györgyi, 1941). If the protein has been precipitated in the form of a thread with some longitudinal alignment of its molecules, the thread shortens and contraction is anisodimensional (A. Szent-Gyorgyi, 1941). If the ATPase activity of the actomyosin thread is suppressed, the addition of ATP does not bring about contraction, but instead the thread becomes extensible (H. H. Weber, 1951). It can now be stretched quite easily, whereas in the absence of ATP it resists extension. ATP, when it is not split, therefore seems to "plasticize" an actomyosin thread; contraction of the thread seems to be specifically associated with the *splitting* of ATP.

A glycerol-extracted muscle, like an actomyosin thread, is inextensible. During the process of extraction, ATP and its precursors have been removed from the muscle. A glycerol-extracted muscle contracts when it is treated with ATP, but if its ATPase activity is suppressed, it becomes extensible (H. H. Weber, 1951).

Actomyosin threads and glycerol-extracted muscles can also be made extensible by treatment with pyrophosphate (Portzehl, 1952), which does not induce contraction. Pyrophosphate dissociates a solution of actomyosin into actin and myosin (Mommaerts, 1948) and these remain separate until the pyrophosphate ions are removed.

All these observations on actomyosin systems *in vitro* suggest that actomyosin will form *in vivo* in the absence of ATP and will dissociate

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Figs. 25–28. Electron micrographs of longitudinal sections through rabbit muscles at four different lengths, that in Fig. 28 being about 20% shorter than that in Fig. 25. A single sarcomere is shown in each case. The two arrays of thin filaments are separated from each other in the slightly stretched muscle (Fig. 25); as the muscle contracts, they move nearer together (Figs. 26 and 27) until they meet (Fig. 28) Magnification × 93,000.

in its presence. The sites of linkage between actin and myosin molecules are presumably located where the lateral projections on the myosin filaments touch the actin filaments. With the linkages locked (no ATP), the fibril would be inextensible; with the locks open (ATP present and ATPase activity suppressed), the arrays of actin filaments could be pulled out of the A bands, and the muscle would be extensible.

A living relaxed muscle is extensible, and it contains ATP. Therefore it is very probable that ATP is able to dissociate the cross-linkages between actin and myosin filaments in the living muscle, and that the ATPase activity of the myosin molecules is suppressed. The low heat output of resting muscle supports the view that ATPase activity is low. Recently, Marsh (1951, 1952) has discovered that muscles contain a "relaxing factor"; this will not only inhibit the ATPase activity of actomyosin or isolated myofibrils, but will also cause glycerol-extracted muscles to become extensible in the presence of ATP (Bendall, 1953, 1954; Hasselbach and Weber, 1953). It is thought that the relaxing factor is active in a relaxed muscle and is inactivated when the muscle is stimulated to contract.

The earliest sign of physical change in an active muscle is the rapid development of resistance to stretch (Hill, 1949c); at about the same time, heat production increases (Hill, 1949b). This strongly suggests that very soon after stimulation, ATP is dephosphorylated and actomyosin forms in the fibrils by cross-linkage of the filaments.

Although an active muscle is relatively inextensible, it is not locked in one position, like a muscle in rigor; it shortens and exerts tension. Mechanisms by which this may be done have been suggested and discussed by A. F. Huxley and Niedergerke (1954), H. E. Huxley and Hanson (1954), Hanson and Huxley (1955), H. E. Huxley (1956, 1959), A. F. Huxley (1957), and H. H. Weber (1958).

B. Hypothetical Schemes for Contraction

Observations which have already been described indicate that when a muscle contracts, its actin filaments "slide" past its myosin filaments. The extent of this sliding movement is much greater than the separation of the lateral projections on the myosin filaments, and it therefore follows that these cannot remain attached to the same points on the actin filaments for more than a very small fraction of the total contraction. Thus in a contracting muscle it seems likely that a repetitive cyclic process goes on at each actin-myosin cross-linkage site, the link being

connected for one part of the cycle and disconnected for the rest. The discussion of actomyosin systems given above suggests that the opening and closing of the links corresponds to the binding and dephosphorylation of ATP. Each time one cycle of operation of the links takes place, the actin filaments are forced to slide a short distance past the myosin filaments in the direction of the center of the sarcomere. When a load on the muscle opposes this movement, tension is exerted. Each link will act many times while the muscle is active.

Two ways in which such a system might work can be suggested. The more obvious one is by a repetitive change in the positions of the lateral projections on the myosin filaments, in such a way that while they are attached to the actin filaments, they exert a tension and draw these filaments along in the direction of the center of the sarcomere; while they are detached from the actin filaments, they return to their original positions; then they attach themselves to the actin filaments at new points. The cycle is then repeated. Change in position of the lateral projections might occur as a result of the binding of ATP to the protein, or as a result of the dephosphorylation of ATP, or as a result of combination between myosin and actin, or (A. F. Huxley, 1957) as a result of thermal motion. The essence of the system is that each link can exert a tension whilst moving along a certain distance during one phase of each cycle of operation, and that these tensions, generated at many links in the muscle, add together in parallel. The distance of movement will necessarily be controlled by the exact spatial arrangement of the cross-links. The total movement of the actin filament while the muscle is shortening is related to the sum of all the movements of any one link while it is connected to the actin filament[3], and the total tension at any particular time is the sum of the tensions generated at all the links in operation.

Another way in which such a system might operate is as follows. Let us suppose that the axial repeating period of myosin-binding sites on the actin filaments is slightly less than the axial repeating period of actin-binding sites on the myosin filaments. Let us also suppose that when cross-links form, the forces involved are sufficient to stretch (or even to break) the actin filaments, and that when actin becomes dissociated from myosin (by ATP), the actin filaments revert to their

[3] It is in fact the average total movement of any one link while it is connected to the actin filament, *divided* by the fraction of the total number of links which are connected at any one time.

original condition and length. If we begin the cycle with actin and myosin combined, and detach the actin filaments in each half of the A band by a wave of detachment sweeping along the A band from its end inwards, then the actin filaments will be drawn into the A band as they shorten to their "free lengths"; as a result, the terminal actin-binding sites on each myosin filament will now be near to new myosin-binding sites on the actin filaments around it; a wave of reattachment now takes place and the system reverts to its original condition, with the difference, however, that the whole of each actin filament has moved along by one "step." In this system, the length changes produced by all the links add together, and each of them need be only a few Angstrom units. Tension is exerted as a result of the changes in the actin filaments.

In the first of these two systems, the myosin molecules change their shapes, and in the second, the actin filaments. Such changes would be difficult to detect; they occur *during* contraction, and no sign that they have taken place remains at the end of contraction. Studies on the effect of ATP on myosin in solution have so far been unrewarding, and no structural alterations have been detected (H. H. Weber, 1950; Gergely, 1956). Experiments on actin, however, have proved very interesting, but difficult to interpret (see Volume II, Chapter I); they have shown that ATP is implicated in the interactions between actin molecules, and it is clearly very important that these should be studied more closely.

The general theory we have outlined goes on to suggest that a muscle remains active for as long as ATP is being split, each of the cross-links going through one cycle of operation each time it captures a "quantum" of ATP and splits it. If the supply of ATP fails, the links lock and remain locked (rigor). If the dephosphorylation of ATP ceases while the supplies of ATP are still maintained, the muscle relaxes.

C Quantitative Aspects of the Mechanism

It is important to see if the mechanism we have been discussing is practicable from a quantitative point of view. In the first instance, let us consider a muscle which is shortening very slowly under a load which it can just lift, i.e. a muscle which is exerting almost its maximal tension, P_0, which we will take to be 3 kg. per cm.^{2} of its cross-section. Under these conditions, the energy liberated as work will be about 3×10^5 ergs per cm.^{2} for each centimeter of shortening. An active muscle also liberates heat. Part of the heat appears whether the muscle

shortens or not, and this part is known as the "activation heat" in a twitch, and the "maintenance heat" in a tetanus. If the muscle shortens, additional heat is liberated, and this is called the "heat of shortening." The amount of shortening heat is proportional to the distance the muscle shortens and is independent of the load being lifted. The total extra energy (work plus shortening heat) liberated by a muscle shortening under nearly maximal load will be about 3.35×10^6 ergs per cm.^{2} per centimeter of shortening. If this energy is derived from the hydrolysis of ATP, which we will take to yield 10,000 calories per mole, then it is easy to show that in the model we have described, one molecule of ATP will have to be split, at each cross-linkage site, for each 120 A. of movement of an actin filament past a myosin filament. This figure agrees well with the "step distance" we would expect on structural grounds.

It is also important to see if the known enzymatic activity of myosin is adequate to release energy at the required rate. If the heat of shortening is 350 g. cm. per cm.^{2} per centimeter of shortening (Hill, 1949a) and if the maximal velocity of shortening is ten muscle lengths per second, then the maximal rate of energy liberation, which will occur when the muscle is shortening under zero load, will be about 3.5×10^6 ergs per second per cm.^{3}. (Maintenance heat—about 1×10^5 ergs—can be neglected here.) On the same basis as before, this is equivalent to the splitting of 5×10^{18} molecules of ATP per second. We know that one cm.^{3} of muscle contains about 5×10^{16} cross-linkage sites; thus each site would have to split about 100 molecules of ATP per second. We have estimated earlier (see p. 204) that each site is associated with two molecules of myosin. *In vitro*, one gram of myosin can split at least 8.3×10^{-5} moles of ATP per second (Mommaerts and Green, 1954). If the molecular weight of myosin is taken to be 424,000, than one molecule will dephosphorylate 35 molecules of ATP per second. Hence one cross-linkage site would be capable of splitting 70 molecules per second. This is reasonably close to what is required of it *in vivo*.

D. Some General Features of the Mechanism

1. *Variation of Energy Release and Tension with Velocity of Shortening*

When a muscle is shortening rapidly, it exerts a lower tension, and the total energy released for a given distance of shortening is less than when it is shortening slowly. We do not know if the lower tension results from a reduction in the tension generated by each cross-link, or if it

results from a reduction in the number of cross-links that are formed, or from both of these possible factors. However, it seems very plausible that a decrease in the number of links will occur when the velocity with which actin filaments slide past myosin filaments is increased, because the time available for a given site on a myosin filament to interact with a given site on the actin filament moving past it will be reduced. If the actin filaments, when linked to the myosin filaments, can only move for a certain distance before the links are broken, then it follows that higher velocities of shortening will lead to a decrease in the number of links present at any given moment, and hence to a decrease in tension (and also in the total energy liberation for a given distance of shortening).

The empirical relationship between tension and *rate* of energy liberation can be derived from the Hill equation (Hill, 1938) defining the relation between tension (P), velocity of shortening (v), and maximal isometric tension (P_o), in terms of two constants, a and b:

$$(P + a)\, v = (P_o - P)\, b$$

It is found that the constant a in this equation is numerically equal to the heat of shortening; thus the rate of energy liberation (over and above maintenance heat) is equal to $(P_o - P)\, b$. Thus, empirically, the rate *increases* as the tension decreases. This is by no means incompatible with the existence of a smaller number of cross-linkages at any given time, for the rate of turnover of links will increase with the velocity of shortening. In fact, the mechanism we have been describing provides a reasonable explanation of the particular form of the relationship between load and rate of working. The probability, per unit time, of a given site on a myosin filament interacting with *any* of a series of attachment points on an actin filament moving past it, is likely to be insensitive to the velocity of the movement; thus the total rate of attachment will be proportional to the number of myosin sites available, which will in turn be proportional to $(P_o - P)$, for the number of occupied sites is proportional to P, and the total number of sites to P_o.

2. *Heat of Shortening*

This type of mechanism does not in itself indicate where the heat of shortening originates. And, indeed, the fact that this heat is largely independent of load is very difficult to account for in any straightforward way. The muscle behaves as though shortening were always

opposed by a constant internal force. Simple friction cannot provide an explanation, for then there should be a difference, equal in amount to 2 a, in the maximal load against which a muscle can shorten, and the minimal load required to stretch it. This is not found to be the case; an active muscle begins to extend as soon as the load on it exceeds the maximal load against which it shortens. Viscous forces, on the other hand, would be expected to vary with the velocity of shortening, and hence with load.

Alternatively, one might suppose that the heat of shortening is simply a part of the energy liberated by the chemical reactions which occur when the muscle is allowed to shorten, a part which is not available as work. However, we have seen that the amount of chemical reaction which occurs appears to be controlled by the amount of work done, so that if a constant fraction of the energy produced in each reaction were liberated as heat, the shortening heat should vary with load; this is not the case. To account for the heat of shortening as a by-product of such reactions, one must therefore have a mechanism in which the fraction of the energy, *per reaction*, which appears as heat, varies with the speed of shortening. The system described by A. F. Huxley (1957) has this general property, and will in fact give good agreement with the observed heat production when appropriate values are given to a small number of adjustable constants. However, serious difficulties still remain when one tries to account for the reported constancy of shortening heat at different muscle lengths (Abbott, 1951) where maximal tension (P_o) has different values.

3. *Variation of Tension with Muscle Length*

The maximal tension that an active muscle can develop varies according to the length of the muscle, in such a way that it is greatest when the muscle is at about the maximal length it can reach in the body, and decreases at shorter and longer lengths. It was pointed out by Huxley and Niedergerke (1954) that this behavior would be expected from the sliding filament model, for when a muscle is stretched, the number of possible cross-linkages will diminish as the extent of overlap between myosin and actin filaments is decreased. However, when the muscle shortens below a certain length, the extent of overlap between the filaments does not change until contraction bands form; this begins to occur at about 65% of resting length, but by then, the tension developed in a frog sartorius muscle, for example, has already

results from a reduction in the number of cross-links that are formed, or from both of these possible factors. However, it seems very plausible that a decrease in the number of links will occur when the velocity with which actin filaments slide past myosin filaments is increased, because the time available for a given site on a myosin filament to interact with a given site on the actin filament moving past it will be reduced. If the actin filaments, when linked to the myosin filaments, can only move for a certain distance before the links are broken, then it follows that higher velocities of shortening will lead to a decrease in the number of links present at any given moment, and hence to a decrease in tension (and also in the total energy liberation for a given distance of shortening).

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2. *Heat of Shortening*

This type of mechanism does not in itself indicate where the heat of shortening originates. And, indeed, the fact that this heat is largely independent of load is very difficult to account for in any straightforward way. The muscle behaves as though shortening were always

problem of how chemical energy is transformed into mechanical work is much more difficult, and it is probably true to say that so far we have merely succeeded in localizing the phenomenon in the cross-bridges.

If the cross-bridges to any particular actin filament work in parallel, then they must move a distance of 50–100 A. during each cycle of operation. This movement is associated with the splitting of one molecule of ATP per bridge. The distance involved is very large compared with the range of a chemical bond, which would exert a much larger force over a much smaller distance. To produce the required excursion, some type of lever or other magnifying system is necessary (for example the "bimetallic strip" model of A. Szent-Gyorgyi, 1947). This is a general difficulty encountered in any contraction mechanism where a substantial number of tension-generating sites on a given filament act *in parallel*. Such difficulties do not arise in a mechanism where the movements produced by the tension-generating sites add up *in series* along a given actin filament, for then the movement per site can be quite small—one or two Angström units. However, it is not at all easy to envisage a mechanism of this type, in which the amount of energy release is determined by the work done, rather than merely by the distance of shortening.

An indirect means of overcoming the difficulty of the large movement required, per molecule of ATP split, is to make use of Brownian motion to bring together two sites which are normally separated by too large a distance for reaction or combination to occur (see, for instance, H. E. Huxley, 1952). This has been done in a very ingenious way in the mechanism of A. F. Huxley (1957). A cross-bridge (or "side-piece") vibrates to and fro in a longitudinal direction on two elastic connections, as a result of thermal agitation, until it comes near to an active point on the actin filament. Having been brought close enough in this way for short-range forces to come into operation, the side-piece joins firmly on to the actin filament. Tension is then exerted by the stretched elastic connection, and work will be done as this shortens back to its resting length, pulling the side-piece and the actin filament along with it. In order to make the system unidirectional, it is postulated that combination with actin can take place only when the side-piece is at one side of the equilibrium position; the combination is broken by ATP at a high rate at the other side of the equilibrium position. The immediate source of energy for contraction is the thermal motion of the side-pieces, stored as potential energy in the elastic con-

fallen to practically zero. The reason for this fall is unknown; possibly the filaments move further apart as the muscle shortens (if the fibril volume remains constant) and as a result the cross-links may operate less effectively.

A muscle which shortens very little in the body will not need long I bands, and indeed would probably be most efficient if the length of the I band were just sufficient to allow shortening to proceed until the Z line reached the A bands, but no further. The indirect flight muscles of *Calliphora* seem to be constructed on this principle (Hanson, 1956). The frog sartorius muscle shortens to a much greater extent, and has relatively long I bands; it appears that this is generally true of vertebrate skeletal muscles. It will be interesting to examine other muscles from this point of view, and also to see if the length at which a muscle develops maximal isometric tension is always about the same as the length at which the maximal number of cross-links can operate.

4. *Biochemical Interpretation of Variation of Energy Production with Load*

It is implicit in this type of mechanism that the enzyme site on the myosin filament is able to split a molecule of ATP more rapidly if cross-linkage to the actin filament has occurred; it is this specific feature which enables the release of energy to be controlled by the amount of external work which is done, and which therefore enables the muscle to operate reasonably efficiently. It is interesting to find that there seems to be some counterpart to this in the behavior of myosin ATPase *in vitro*. Experiments which are discussed in Volume II, Chapter I show that the ATPase activity of myosin is low when actin is absent and is greatly increased when actin is present, provided that combination between the two proteins can occur. This suggests that the activity of an enzyme site on a myosin filament is increased when a link to an actin filament has been formed at some point in the cycle of events taking place each time the site reacts.

5. *The Mechanism of Energy Transfer*

The two most important features of muscular contraction are that the contractile mechanism is able to transform the energy liberated in the chemical reaction into mechanical work, and that the amount of mechanical work which is done controls the extent of the chemical reaction which takes place. We have seen that experimental evidence indicates how the second of these two features may possibly be achieved. The

exactly what process at the cross-bridges causes the two sets of filaments to slide past each other. On the other hand, it does provide a means by which arguments based on physiological and biochemical information about muscles and muscle proteins can be brought to bear on this so intractable question, and can be used to define, more and more closely, the nature of this detailed process. And it is reasonable to hope that one will eventually be able to embody these data in a unique and verifiable hypothesis.

References

Abbott, B. C. (1951). *J. Physiol. (London)* **112**, 438.
Amberson, W. R., Smith, R. D., Chinn, B., Himmelfarb, S., and Metcalf, J. (1949). *Biol. Bull.* **97**, 231.
Astbury, W. T. (1947). *Proc. Roy. Soc.* **B134**, 303.
Astbury, W. T. and Dickinson, S. (1940). *Proc. Roy. Soc.* **B 129**, 307.
Astbury, W. T., and Spark, L. C. (1947). *Biochim. et Biophys. Acta* **1**, 379.
Astbury, W. T., Reed, R., and Spark, L. C. (1948). *Biochem. J.* **43**, 282.
Bailey, K. (1956). *Pubbl. staz. zool. Napoli* **29**, 96.
Bear, R. S. (1944). *J. Am. Chem. Soc.* **66**, 2043.
Bear, R. S. (1945). *J. Am. Chem. Soc.* **67**, 1625.
Bear, R. S., and Selby, C. C. (1956). *J. Biophys. Biochem. Cytol.* **2**, 55.
Bendall, J. R. (1953). *J. Physiol. (London)* **121**, 232.
Bendall, J. R. (1954). *Proc. Roy. Soc.* **B142**, 409.
Bennett, H. S., and Porter, K. R. (1953). *Am. J. Anat.* **93**, 61.
Cohen, C., and Hanson, J. (1956). *Biochim. et Biophys. Acta* **21**, 177.
Corsi, A., and Perry, S. V. (1958). *Biochim. J.* **68**, 12.
Davies, H. G., Wilkins, M. H. F., Chayen, J., and La Cour, L. F. (1954). *Quart. J. Microscop. Sci.* **95**, 271.
Draper, M. H., and Hodge, A. J. (1949). *Australian J. exptl. Biol. med. Sci.* **27**, 465.
Farrant, J. L, and Mercer, E. H. (1952). *Exptl. Cell. Research* **3**, 553.
Finck, H., Holzer, H., and Marshall, J. M. (1956) *J. Biophys. Biochem. Cytol.* **2**, *Suppl.* 175.
Gergely, J. (1956). *J. Biol. Chem.* **220**, 917.
Gergely, J., Gouvea, M. A., and Karibian, D. (1955). *J. Biol. Chem.* **212**, 165.
Hall, C. E., Jakus, M. A., and Schmitt, F. O. (1946). *Biol. Bull.* **90**, 32.
Hanson, J. (1956). *J. Biophys. Biochem. Cytol.* **2**, 691.
Hanson, J., and Huxley, H. E. (1953). *Nature* **172**, 530.
Hanson, J., and Huxley, H. E. (1955). *Symposia Soc. Exptl. Biol.* **9**, 228.
Hanson, J. and Huxley, H. E. (1957). *Biochim. et Biophys. Acta* **23**, 250.
Hanson, J., Lowy, J., Huxley, H. E., Bailey, K., Kay, C. M., and Ruegg, J. C. (1957). *Nature* **180**, 1134.
Hasselbach, W. (1953). *Z. Naturforsch.* **8b**, 449.
Hasselbach, W., and Schneider, G. (1951). *Biochem. Z.* **321**, 462.
Hasselbach, W., and Weber, H. H. (1953). *Biochim. Biophys. Acta* **11**, 160.
Haswell, W. A. (1889). *Quart. J. Microscop. Sci.* **30**, 31.
Hill, A. V. (1938). *Proc Roy. Soc.* **B126**, 136.
Hill, A. V. (1949a). *Proc. Roy. Soc.* **B136**, 195.
Hill, A. V. (1949b). *Proc. Roy. Soc.* **B136**, 242.
Hill, A. V. (1949c). *Proc. Roy. Soc.* **B136**, 399.

nections, but the system does not violate the second law of thermodynamics because energy has to be supplied to break the actin-myosin linkage in another part of the cycle.

There is very little experimental evidence at present to help to decide how (or whether) movement of the cross-bridges takes place. Pure myosin is reported (H. H. Weber, 1950; Gergely, 1956) to be unaffected in its sedimentation constant, viscosity, and light-scattering behavior by the presence of ATP. However, changes in molecular shape which could considerably alter the positions of the cross-bridges on the filaments might not be easy to detect in isolated molecules in solution. Also, it may be possible that a shape change only occurs when combination with actin is able to take place.

In contrast to its apparent lack of effect on myosin, ATP seems to be involved in at least two important structural reactions of actin molecules, and it would be surprising if these did not play some part in muscular contraction. The monomer-dimer transformation of actin, which Tsao (1953b) has shown to take place instantaneously when ATP is added to actomyosin, is a very striking phenomenon; and the dephosphorylation of bound ATP which accompanies the G to F transformation of actin (see Volume II, Chapter I) could also provide a link between chemical and mechanical effects. One could speculate that if these molecular transformations, or equivalent ones *in vivo*, produced small changes in the length of an actin filament, it might be possible to make this filament crawl along the system of cross-bridges from the three myosin filaments around it.

VII. CONCLUSIONS

The purpose of this chapter was to see to what extent our present knowledge of the molecular structure of cross-striated muscle can illuminate the relation between the physiological and biochemical properties of the tissue, and can provide information about the detailed events taking place when the chemically stored energy, through the mediation of an enzyme system, is converted into mechanical work. The sliding filament model, and its detailed dimensions, now seem fairly well established. This model is able to give a logical and straightforward account of many aspects of the general physiological behavior of striated muscle in terms of its chemical constituents and their known properties and reactions. The account is, however, incomplete in perhaps its most important and fundamental detail, for it does not specify

Szent-Gyorgyi, A., Mazia, D., and Szent-Gyorgyi, A. G. (1955). *Biochim. et Biophys. Acta* **16**, 339.
Szent-Gyorgyi, A. G. (1951a). *Arch. Biochem. Biophys.* **31**, 97.
Szent-Gyorgyi, A. G. (1951b). *J. Biol. Chem.* **192**, 361.
Szent-Gyorgyi, A. G. (1953). *Arch. Biochem. Biophys.* **42**, 305.
Tsao, T. C. (1953a). *Biochim. et Biophys. Acta* **11**, 227.
Tsao, T. C. (1953b). *Biochim. et Biophys. Acta* **11**, 236.
Tsao, T. C., Bailey, K., and Adair, G. S. (1951). *Biochem. J.* **49**, 27.
Velick, S. F. (1956). *Biochim. et Biophys. Acta* **20**, 228.
Weber, A. (1956). *Biochim. et Biophys. Acta* **19**, 345.
Weber, H. H. (1950). *Proc. Roy. Soc.* **B137**, 50.
Weber, H. H. (1951). *Z. Elektrochem.* **55**, 511.
Weber, H. H. (1958). "The Motility of Muscle and Cells". Harvard University Press.

Hodge, A. J. (1955). *J. Biophys. Biochem. Cytol.* **1**, 361.
Hodge, A. J. (1956). *J. Biophys. Biochem. Cytol.* **2**, *Suppl.* 131.
Hodge, A. J., Huxley, H. E., and Spiro, D. (1954). *J. Exptl. Med.* **99**, 201.
Hoffmann-Berling, H., and Kausche, G. -A. (1950). *Z. Naturforsch.* **5b**, 139.
Holtzer, A., and Lowey, S. (1956). *J. Am. Chem. Soc.* **78**, 5954.
Holtzer, H., Marshall, J. M., and Finck, H. (1957). *J. Biophys. Biochem. Cytol.* **3**, 705.
Huxley, A. F. (1957). *Progr. in Biophys. and Biophys. Chem.* **7**, 257.
Huxley, A. F., and Niedergerke, R. (1954). *Nature* **173**, 971.
Huxley, A. F., and Taylor, R. E. (1955). *Nature* **176**, 1068.
Huxley, H. E. (1951). *Discussions Faraday Soc.* **11**, 148.
Huxley, H. E. (1952). PhD. Thesis, Cambridge University.
Huxley, H. E. (1953a). *Proc. Roy. Soc.* **B141**, 59.
Huxley, H. E. (1953b). *Biochim. et Biophys. Acta* **12**, 387.
Huxley, H. E. (1956). *Endeavour* **15**, 177.
Huxley, H. E. (1957). *J. Biophys. Biochem. Cytol.* **3**, 631.
Huxley, H. E. (1959). *In* "The Cell" (J. Brachet and A. Mirsky, eds.), Academic Press, New York (in press).
Huxley, H. E., and Hanson, J. (1954). *Nature* **173**, 973.
Huxley, H. E., and Hanson, J. (1957a). *Biochim. et Biophys. Acta* **23**, 229.
Huxley, H. E., and Hanson, J. (1957b) *Proc. Stockholm Conf. Electron Microscopy, 1956* p. 202.
Huxley, H. E., and Perutz, M. (1951). *Nature* **167**, 1054.
Hyashi, T., Rosenbluth, R., Satir, P., and Vozick, M. (1958) *Biochim. et Biophys. Acta* **28**, 1.
Laki, K., and Carroll, W. R. (1955). *Nature* **175**, 389.
Marsh, B. B. (1951). *Nature* **167**, 1065.
Marsh, B. B. (1952). *Biochim. et Biophys. Acta* **9**, 247.
Middlebrook, W. R. (1958). *Abstr. Biophys. Soc. Meeting Cambridge, Mass.* p. 46.
Mommaerts, W. F. H. M. (1948). *J. Gen. Physiol.* **36**, 361.
Mommaerts, W. F. H. M., and Green, I. (1954). *J. Biol. Chem.* **208**, 833.
Pauling, L., and Corey, R. B. (1951). *Proc. Nat. Acad. Sci. U. S.* **37**, 261.
Pauling, L., Corey, R. B., and Branson, H. R. (1951). *Proc. Nat. Acad. Sci. U. S.* **7**, 205.
Perry, S. V. (1955). *Symposia Soc. Exptl. Biol.* **9**, 203.
Perry, S. V., and Corsi, A. (1958). *Biochem. J.* **68**, 5.
Perutz, M. (1951). *Nature* **167**, 1053.
Philpott, D., and Szent-Gyorgyi, A. G. (1954). *Biochim. Biophys. Acta* **15**, 165.
Portzehl, H. (1952). *Z. Naturforsch.* **7b**, 1.
Pringle, J. W. S. (1957). "Insect Flight." Cambridge Univ. Press, London and New York.
Rosza, G., Szent-Gyorgyi, A., and Wyckoff, R. W. G. (1949). *Biochim. et Biophys. Acta* **3**, 561.
Schmidt, W. J. (1934). *Z. Zellforsch. u. mikroskop. Anat.* **21**, 224.
Selby, C. C., and Bear, R. S. (1956) *J. Biophys. Biochem. Cytol.* **2**, 71.
Sjostrand, F., and Andersson, E. (1956). *Exptl. Cell Research* **11**, 493.
Sjostrand, F., and Andersson, E. (1957). *J. Ultrastructure Research* **1**, 74.
Spiro, D. (1956). *Exptl. Cell Research* **10**, 562.
Straub, F. B. (1942). *Studies Inst. Med. Chem. Univ Szeged* **2**, 3.
Straub, F. B. (1943). *Studies Inst. Med Chem Univ. Szeged* **3**, 23.
Szent-Gyorgyi, A. (1941). *Studies Inst Med. Chem. Univ. Szeged* **1**, 17.
Szent-Gyorgyi, A. (1947). "Chemistry of Muscular Contraction." Academic Press, New York.

CHAPTER VIII

Molecular Structure and Function of Smooth Muscle[1]

ARPAD CSAPO

I. INTRODUCTION

The molecular structure and function of smooth muscle may be considered more meaningfully if compared to that of cross-striated muscle. This approach, both in experiments and in exposition, may not only point to some *common features of structure and function in muscle*, but may also put smooth muscle in its place in the large family of contractile and excitable tissues. It may help us in distinguishing between "fundamental" and "special" properties of muscle, and in setting proper limits upon general validity of new concepts.

Beside stressing the value of comparison, the present article will emphasize the importance of studying the function of muscle at different levels of organization. Arguments will be restricted to experience with the frog sartorius and toe muscle, the rabbit psoas, the retractor penis of the turtle, the rabbit and the human uterus, and the tenia coli of the guinea pig. The group is small but represents a variety of physiological functions. Each type of muscle will be considered to a limited extent only.

To students of smooth muscle, the support offered by knowledge of

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mechanism of muscular contraction. How well his new theory is supported will become evident from his present article and that of A. G. Szent Gyorgyi. He now believes that the sarcomere consists of a supporting protein structure to which are attached the meromyosins, the heavy portion being in the middle of the sarcomere. How then, is the contractile machinery energized as far from the H-meromyosins as 1000 A.? Szent-Györgyi suggests that electronic excitation is at work here. Somewhat modifying his earlier concept of the final event in muscle contraction as an interreaction between AM, ATP, and ions, he now considers water to be an integral part of the system and not merely a constituent of the milieu.

Hippel *et al.* (1957) question the presence of A in natural AM ("myosin B"), doubting that the reaction of AM with ATP involves "dissociation" and "association" of A and M. They suggest the deformation of M by ATP as the physical change underlying the observed phenomena. Gergely and Kohler (1957), using the same light-scattering technique used by Hippel *et al.*, report however that dissociation and association of A and M indeed occurs during the AM-ATP interreaction. Gergely's laboratory also demonstrated that A is present in natural AM, by extracting A from it (personal communication).

Such uncertainties exemplify the problems which arise in studying muscle function at the molecular level. No doubt these studies promise a penetrating insight into the molecular mechanism of muscle function but the danger of artifacts at the molecular level is indeed great. Because of this danger, interpretations must be cautious and tentative. Intact muscle, if properly handled, is free of artifacts but its complexity frequently permits the investigator to describe phenomena only, rather than to state in biochemical or biophysical terms what happens inside the muscle; thus the component parts of the contractile machinery remain unrevealed as well as their respective nature and interrelations. A. V. Hill stated, for example, in 1956 "It is true that the heat production is unspecific: even the most accurate description of it does not allow us to decide what exactly the underlying chemical events are but it does provide a framework into which these must be fitted."

Uncertainties of this kind and the lability of concepts about structure and function in cross-striated muscle make one wonder whether the time is yet appropriate to consider the molecular mechanism of function in cross-striated and smooth muscle. Appreciating his limitations the author will proceed, nevertheless, in order to fulfill a commitment.

cross-striated muscle has been a comfort; but as this article is written it is apparent that this ground is becoming insecure.

Thus H. E. Huxley (1956) proposed the "sliding" model for the molecular mechanism of muscular contraction, a well documented hypothesis considerably extended by A. F. Huxley (1956, 1957). In spite of the variety of supporting evidence, the validity of the model is questioned on the ground of contradictory findings. A. Szent-Gyorgyi (1958), the senior student of the proteins myosin (M) and actin (A), which are supposed to slide past each other when muscle shortens or lengthens, argued that the model is too mechanical and that it takes no advantage of the drastic physicochemical changes characteristic of the AM-ATP interreaction and does not explain how the "AM-thread," free of organized structure, can shorten 80% of its "resting" length. The general validity of the sliding model may also be questioned since it does not explain shortening in smooth muscle, which exhibits no periodic structure. Sjostrand and Andersson-Cedargren (1957) state that the diameter of the myofibrils in the "A" band increased in proportion to the shortening and that both the "I" and "A" bands shorten when the muscle contracts. They believe that shortening is associated with "folding up or a change in the helical arrangement of the subunits of the myofilaments." The internal coherence of the sliding model also depends on the exact chemical and physical characteristics of M and A, and other muscle proteins which are as yet defined only operationally and without general agreement.

Laki (1957) suggests, for example, that "a comparison of the amino acid composition of tropomyosin and actin shows that myosin and the *meromyosins might be looked upon as compounds of tropomyosin and* actin." This statement implies that M and the meromyosins are artifacts of extraction and preparation rather than genuine constituents of living muscle. In the articles of Gergely and his colleagues (1955; Gergely, 1953), no opinion is expressed about the genuineness of the meromyosins as subunits of the contractile proteins.

Laki's suggestion is objected to in Szent-Györgyi's laboratory where M, A, and the meromyosins are looked upon as genuine units of the contractile machinery. In his recent article, however, Szent-Györgyi (1958) emphasized the genuineness of the meromyosins only, implying that M may be an extraction product. Referring to recent observations of Inoue, Holtzer and Marshall, and A. G. Szent-Gyorgyi, he proposed a rather drastic modification of current concepts about the molecular

(Csapo, 1955) offered only slightly more information than did the light microscope. Myofibrils of about 0.5—1 μ in diameter were seen running

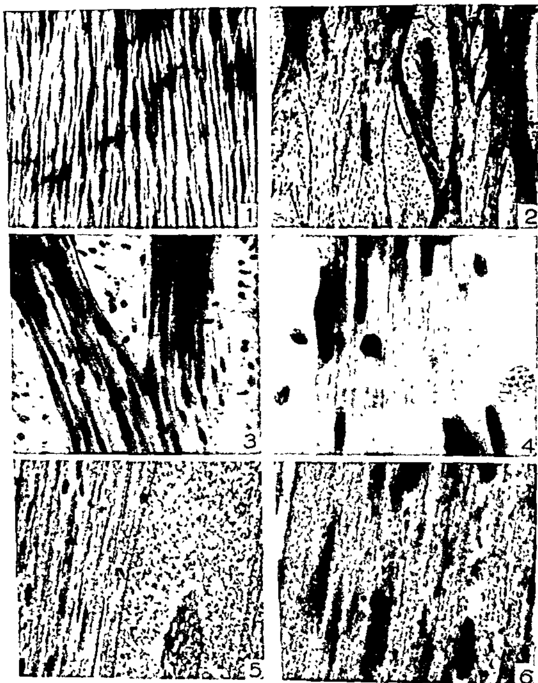


Fig. 1. Structure of the longitudinal muscle layer of the pregnant rabbit uterus at term (Csapo, 1955). Numbers 1–4, light microscopy; Magnification: × 12, 75, 300, and 1,000, respectively; numbers 5 and 6, *electronmicrographs*; Magnification: × 10,000 and 20,000, respectively.

II. "HEADACHE MUSCLE"

Smooth muscles have been more often used to test drug action than to investigate their intrinsic properties. The techniques used in these studies are objectionable, frequently yielding controversial and inconclusive results, and the prejudice developed against them among muscle physiologists is justified to a considerable extent. The widely used phrase "headache muscle" is not altogether inappropriate. An attempt will be made here to limit this prejudice to its proper extent. This is done in appreciation of Hill's statement (1956): "The mechanical response of active muscle is easy to record; indeed too easy, for very inadequate methods have been used and the older physiological literature is burdened with results of experiments which depended more on the properties of levers than on those of muscle." Largely because of a lack of appreciation of the significance of adequate techniques, smooth muscle physiology became an isolated and retarded discipline, conducted without regard to the fundamental similarity of contractile tissues. Special attention, therefore, will be paid here to techniques and to those experiments which suggest that a variety of muscle tissues are alike in the fundamental principles of their function.

III. STRUCTURE

The smooth muscle of the rabbit uterus, like many other smooth muscles, forms a coat around a hollow chamber. Its function is mainly to exert pressure on the contents of the chamber in the process of emptying it. This muscle coat is built, like cross-striated muscle, of long fiber-bundles about 100 μ in diameter. In cross-striated muscle, the single fiber, a giant multi-nucleated cell with an excitable membrane around it, is assumed to be the final physiological unit; in smooth muscle, however, the fiber-bundle of the uterus is composed of a number of individual cells about 10 μ in diameter and 50–300 μ in length, each of which possesses an excitable membrane (Csapo, 1955). This structural difference, however, may be of minor significance. Porter and Palade's (1957) myoplasmic reticulum in cross-striated muscles may turn out to be a membrane—like structure around myofibrils. Bozler's studies (1938) indicate that the fiber bundles of uterine smooth muscle behave as syncytia, responding in an "all or none" fashion and that in a d.c. field the bundles develop polarity. The possibility, therefore, should be examined that fiber bundles in smooth muscles are surrounded by an excitable structure.

The first studies of the myometrial cell with the electron microscope

The significance of ultrastructure in smooth muscle is now increasingly realized and the possibilities offered by a comparative approach, in suggesting generalizations about muscle structure, begin to be appreciated. In this volume Lowy and Hanson, in detail, discuss the structure of smooth muscle. Their published work and that of Weinstein and Ralph (1951), Hodge and associates (1954), Mark (1956), and Kawaguti and Ikemoto (1957) do not yet suggest structural features in smooth muscle which would explain their respective physiological functions. Obviously, more work needs to be done before we can account, in structural terms, for the fact that the rabbit uterus obeys Hill's classical equation.

IV. THE CONTRACTILE PROTEINS

When the "natural" actomyosins of cross-striated and of uterine muscle were first compared (Csapo, 1948, 1949, 1950), only a limited set of techniques were available for analysis. These observations showed striking similarities between the proteins of the two types of muscle. Considerable advancement in techniques has not yet convincingly challenged the original notion that the contractile proteins of these different types of muscle are closely similar.

The statement that the uterus contains actotropomyosin, rather than AM, came from Snellman and Tenow (1954). Considering their extraction procedure Csapo (1955) suggested that most of the AM present in the muscle was left by them in the debris and the character of what little AM they brought into solution was masked by impurities, i.e. proteins extractable by strong salts. Kominz and Saad (1956) studied the question experimentally, finding that Csapo's extraction procedure yielded considerably more AM from the uterus than that of Snellman and Tenow. Furthermore, purified AM yielded a peak in the ultracentrifuge which changed into a hypersharp M peak on the addition of ATP. Thus the ultracentrifuge indicated no difference between the contractile proteins of cross-striated and smooth muscle. Uterine AM repeatedly precipitated, however, shows an additional peak beside that of AM. Thus an impurity in the AM solution which may well be tropomyosin cannot be eliminated by precipitation by dilution. But the presence of tropomyosin in the uterus, or its relatively high concentration, does not in any way interfere with the notion that the actomyosins of different muscles are similar. Recent experiments of Tonomura *et al.* (1957) and Tonomura and Sasaki (1957) also show that smooth muscles contain AM and M but that their tropomyosin con-

parallel to the long axis of the cell, while the space between the myofibrils was filled by mitochondria, arranged like beads in a row. It was not possible to determine where the myofibrils are anchored in the cell,



FIG. 2. Electron micrograph of the rabbit myometrium at term (Csapo, 1955), showing longitudinal structure within the myofibrils and mitochondria between them. Magnification. × 40,000.

i.e., where they begin and end. Although a regular system of longitudinal filaments was clearly visible within the myofibrils, the resolution was not sufficient to show whether the filaments were of one or two types nor could their diameter be measured. Cross-striations, however, were never seen at any magnification.

uterus by A. G. Szent-Györgyi's KI treatment. Such a preparation, after repeated precipitation, sediments as M with a hypersharp peak, and its change in viscosity on ATP addition is negligible. It readily combines with A of cross-striated muscle as indicated by the characteristic rise in viscosity. The optimum combination ratio M: A is 5:1. Such a synthetic AM (a hybrid of cross-striated and uterine muscle) exhibits the well known change in viscosity on the addition of ATP. Studies on such synthetic AM confirm the notion that the drop in viscosity is a measure of the AM concentration. These studies again suggest close similarities between the contractile proteins of different muscles.

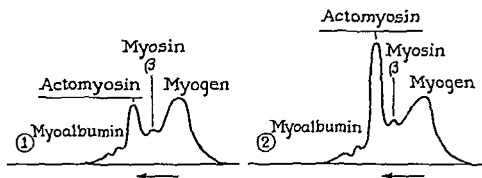


FIG. 4. Electrophoretic patterns of uterine muscle proteins (after Blasius and Schuck, 1955). Number 1, uterus of immature rabbit; 2, the same after estrogen treatment. Note the increase of the AM peak after estrogen treatment.

It has long been demonstrated that the contractile proteins of the uterus are controlled by endocrine regulation. The removal of the ovaries results in a drastic decrease in the AM concentration of the uterus, whereas estrogen substitution therapy restores the *status quo* (Csapo, 1948, 1949, 1950). Under certain physiological conditions, for example, during pregnancy, the AM concentration of the uterus increases in order to meet the functional requirements imposed upon it by parturition. The uterus is not unique, however, in responding to hormone action, as indicated by the changes in AM concentration of chick cross-striated muscle during the hatching period and afterwards during sexual maturation (Csapo and Hermann, 1951). Endocrine regulation of the contractile proteins of cross-striated muscle in the adult animal is not yet obvious, probably because of its complexity. But studies along these lines may well reveal fundamental correlations between regulation and disease. The experiments (Csapo, 1950) demonstrating the influence of estrogen on the contractile proteins of the

centration is higher than that of cross-striated muscle. It appears, therefore, that the ratios of different proteins to one another is different in different muscles, but there is no convincing evidence yet that the contractile proteins themselves are different from one muscle to the next.

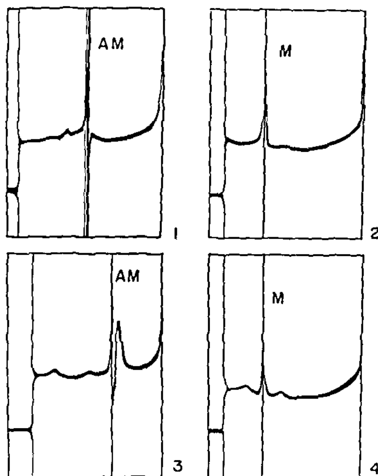


FIG. 3. Ultracentrifuge patterns of actomyosin (AM) and myosin (M) (after Komintz and Saad 1956). Number 1, rabbit skeletal muscle; 2, the same with ATP; 3, human uterus at term; 4, the same with ATP. KCl = 0.5 *M*, $MgCl_2 = 10^{-3}M$, ATP = $5 \times 10^{-3}M$. Time of exposure 64–114 minutes after reaching 59, 780 r.p m. Protein concentration = 0.7–0 9%. Sedimentation rates: 1, 8.5; 2, 3.9; 3, 9.3; 4, 4 4. Note the characteristic hypersharp M peaks in the presence of ATP.

Extraction of rabbit cross-striated muscle for a short time yields M (plus the debris containing A) whereas long extraction yields AM. Extraction of uterine muscle always yields AM and heretofore nobody has obtained pure M or A from the uterus. Recently, however, Nagy and Csapo (unpublished observation) obtained A-free M from the

uterus by A. G. Szent-Györgyi's KI treatment. Such a preparation, after repeated precipitation, sediments as M with a hypersharp peak, and its change in viscosity on ATP addition is negligible. It readily combines with A of cross-striated muscle as indicated by the characteristic rise in viscosity. The optimum combination ratio M: A is 5:1. Such a synthetic AM (a hybrid of cross-striated and uterine muscle) exhibits the well known change in viscosity on the addition of ATP. Studies on such synthetic AM confirm the notion that the drop in viscosity is a measure of the AM concentration. These studies again suggest close similarities between the contractile proteins of different muscles.

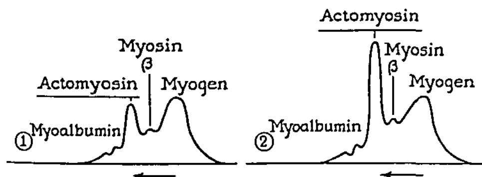


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uterus have been successfully repeated and extended by Blasius and *Schuck (1955) and by Cretius (1957).*

The information obtained through these experiments allowed us to

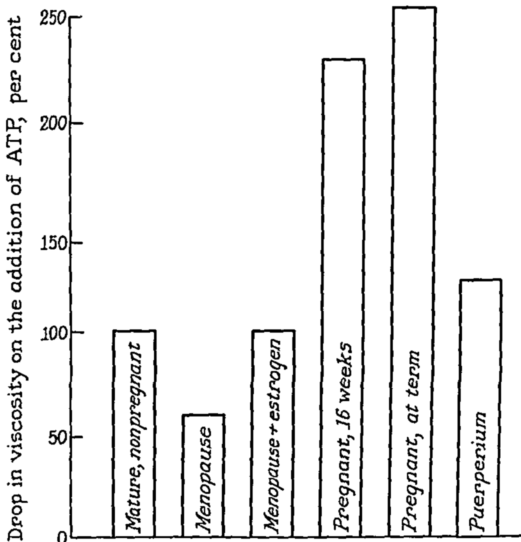


Fig. 5. Actomyosin of the human uterus in different endocrine conditions (after Cretius, 1957). Note the decrease in AM after withdrawal of estrogen and its increase on estrogen administration, and also the further increase in AM during pregnancy and its decrease after parturition.

alter the AM concentration of the uterus at will and to study the question of whether the working capacity of the muscle is determined by its AM concentration. Experiments (Csapo and Corner, 1953) showed that maximum isometric tension falls with decreasing AM concentration and rises when the latter is increased, leaving tension/[AM]

constant. With synthetic AM formed by varying the A concentration in vitro, Hayashi *et al.* (1956) arrived at the same conclusion. Tension in living muscle, of course, does not depend upon the [AM] only.

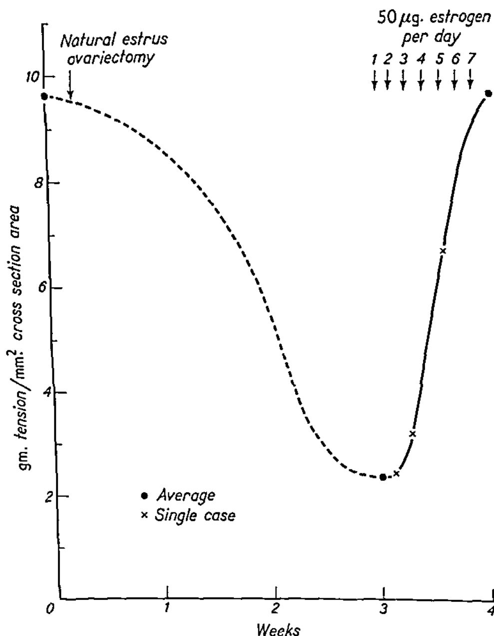


FIG. 6. Effect of estrogen on the maximum tension of the rabbit uterus. (Csapo and Corner, 1953). Note decrease in tension following ovariectomy and its return to the original (estrous) value after estrogen treatment. Tension in natural estrous = 9.6 g. ± 1.11 SE per mm^2. cross section area; the [AM] = 7.6 mg. per gram of tissue After ovariectomy, tension = 2.3 g. ± 0.41 SE per mm^2. the [AM] = 1.7 mg. per gram. Thus tension/[AM] ratio remains constant in the two different endocrine states: 1.27 and 1.35, respectively.

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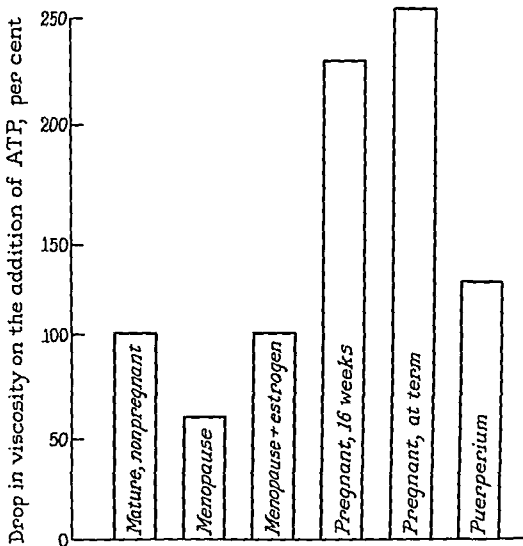


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the speed of shortening, so that the difference in the kinetics of shortening disappears, is only a puzzle as yet. Tonomura and Sasaki (1957) noted the interesting fact that the AM of esophagus smooth muscle has a "functional unit weight" of 10×10^{-5} g., whereas that of the psoas muscle 1.85×10^{-5} g. Functional unit weight is the weight of the protein which combines with 1 molecule of ATP.

The presence of the high energy phosphate (~ph) compounds ATP and creatine phosphate (CP) in the uterus was already known to Lohmann (1934). The nonpregnant estrogen-dominated rabbit uterus contains 1.9–2.4 μM ATP per gram and 0.74–1.26 μM CP per gram of tissue (Csapo and Gergely, 1950; Menkes and Csapo, 1952). Cretius (1958) reports similar data for the human uterus and Born (1956) for the tenia coli of the guinea pig. From these observations, it appears that the ~ph concentration of smooth muscle (2.6–3.6 μM per gram) is about 5–7 times less than that of cross-striated muscle (17.5–25 μM per gram). This difference may have considerable functional significance. It is known that excitability depends on the membrane potential "excess" over a critical value (Jenerick and Gerard, 1953). It is also known that the excess potential depends on the [CP], as shown by Ling (1952). In smooth muscles one would expect (from the low [CP]) a lower membrane potential, and a tendency toward activity and contracture. In fact, these are characteristic properties of a variety of smooth muscles, as will be pointed out later. The low ~ph concentration in the uterus, as compared with cross-striated muscle, coincides with a proportionally lower [AM], resulting in similar ratios of AM: ~ph in both types of muscle. The similarity in AM: ~ph ratios again suggest that these compounds are parts of the same functional system, serving the same purpose in contractility.

The notion that ATP is the immediate source of energy originates from the work of Lundsgaard (1930, 1934) and Lohmann (1934). The former demonstrated that frog muscle relying entirely on its stored ~ph (poisoned by IAA + N_2) can perform only a limited number of contractions and that contractions cease when the ~ph (ATP + CP) are exhausted. The Lohmann reaction (ADP + CP $\rightleftarrows$ ATP + C), on the other hand, indicates that CP will disappear before stored ATP is hydrolyzed.

Lundsgaard's experiments have been successfully repeated with the uterus (Csapo and Gergely, 1950; Csapo, 1955). A recent reconsideration of Lundsgaard's original experiment, however, brought out some

Factors influencing excitability and energetics may completely suspend muscle function without altering the [AM] at all. These possibilities are utilized in the regulation of muscle function as will be mentioned later.

Needham and Cawkwell (1957), using non-collagenous nitrogen as a measure of contractile proteins in different fractions of rat uterus, could confirm the changes in [AM] only after ovariectomy and subsequent estrogen treatment but not during pregnancy. This technique may be less specific for AM than the "ATP-sensitivity" technique. Let us suppose that both the nonpregnant and pregnant uterus contains 10 mg. M per gram of tissue but that the nonpregnant has 1 mg. A per gram and the pregnant has 2 mg. A per gram of uterus. The sum M + A will be 11 mg. per gram and 12 mg. per gram tissue, respectively, an insignificant difference indeed. However, the [AM] in the nonpregnant uterus (taking 5:1 ratio of M: A) will be 6 mg., whereas in the pregnant uterus [AM] will be 12 mg.—a 100% increase. Viscometry suggests that it is the [A] which increases significantly during pregnancy.

It may be concluded from these observations that the contractile proteins M and A are similar in different muscles although their respective concentrations are different. Also variable are their ratios to other muscle proteins, which perhaps provide a framework only, or may actually participate, in some yet unknown way, in the contraction cycle. Similarities in the contractile proteins of different muscles are encouraging. They suggest that a variety of muscles obey certain fundamental principles in function. If so, we have to understand these principles first to appreciate modifications, in the complexity of muscle function, which have developed during the process of evolution in order to meet special functional requirements.

V. ENERGETICS

The above considerations prompt us to look for similarities in energetics in cross-striated and smooth muscle. Studies with uterine AM-threads (Csapo, 1948, 1949, 1950) showed that the thread contraction phenomenon occurs with uterine muscle proteins, as well as with those of cross-striated muscle. The fact that shortening is considerably slower with uterine AM than with threads prepared from cross-striated muscle may be significant. The observation that a water soluble protein ("X-factor") of cross-striated muscle added to uterine AM-threads increases

cles develop a set of tetani, of somewhat reduced amplitude, when stimulated in a strong electric field. Contractility is lost when ATP is exhausted. These and other experiments to be referred to later, emphasize the significance of the distinction between excitability and contractility. They illustrate that muscles which cease to be "excitable" may remain contractile and that the question of "membrane" and "myoplasmic" excitability must be thoroughly investigated.

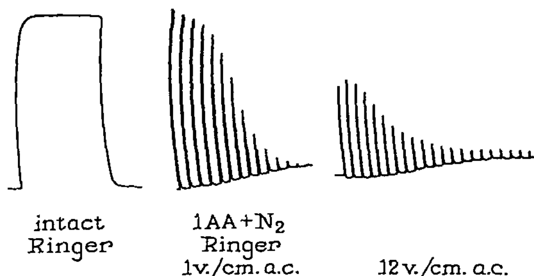


FIG. 8. The "second phase" of tension development in the IAA- and N_2-blocked sartorius in a strong electric field (Csapo, 1957). Note that the muscle only becomes "inexcitable" with the usual tetanic stimulus (1 v. per centimeter) but remains contractile for a short period if stimulated in a strong (12 v. per centimeter) electric field.

Munch-Petersen (1953) and Lange (1955) claimed to have obtained direct evidence that contracting muscle contains less ATP than its "resting" control, implying that the bond energy of ATP is utilized during contraction. Fleckenstein and his colleagues (1954) and Mommaerts (1954), on the other hand, claimed that a single muscle twitch can occur without ATP breakdown and proposed that ATP is not the immediate source of energy in muscle contraction. Needham (1956) has appropriately dealt with this question in a recent article. A few additional remarks will suffice here (see also Volume II, Chapter II).

In the majority of experiments, the muscle is frozen in order to preserve it for chemical analysis. This quick freezing complicates the

significant points (Csapo and Gergely, unpublished). First it was found that repeatedly tetanized frog or uterine muscles, blocked by IAA + N_2 have exhausted only their CP when they cease to contract, while their [ATP] was slightly changed. If ATP provides the contractile energy,

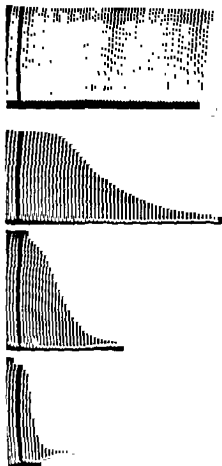


FIG. 7. Maximum tension as a function of the ~ph content of the rabbit uterus (Csapo, 1955) Four identical segments from the same rabbit uterus, in natural estrus, are tetanized once every minute. From top to bottom: 1, Krebs solution, 95% O_2 + 5% CO_2; 2, 1/10,000 *M* IAA is applied, 3, O_2 off, N_2 on; 4, IAA + N_2. Note that tension declines more rapidly as the resynthesis of ~ph is increasingly blocked.

why should the muscle cease to contract with a practically unchanged store of ATP? Further experiments showed that muscles under these conditions become only "inexcitable," but indeed remain contractile. They will not respond to a conventional tetanic stimulus, probably because with the loss of CP the membrane mechanism for propagated activity is eliminated. In spite of their loss of CP, however, these mus-

tension, after quick releases to zero tension, at different times during a 5-sec. tetanic stimulus. He found that the increase in rate may be as much as tenfold, indicating that activation is a gradual process, taking several seconds for completion. Other muscles (retractor penis and retractor capitis collique of the turtle) occupy a position between the rabbit uterus and the frog sartorius, which shows an increase in rate as small as 10%. This difference between cross-striated and smooth muscles in the kinetics of the development of the active state is characteristic and significant in the interpretation of the "active state".

It is stated (Wilkie, 1956) that the physical state in muscle is the same during the early part of a twitch and in a tetanus, activity being maximal in both cases. A single stimulating pulse sets off the "active state" fully without maintaining it. Thus the active state declines before maximum tension is reached. The result is a twitch. If a series of pulses are placed close enough to one another (30 per second for the frog sartorius at 0°C.), the active state is maintained and individual twitches fuse into a smooth tetanus. Both twitches and tetani can be obtained in the uterus, as well as all the intermediate values of tension due to the "summation" effect. It is doubtful that activation is maximal in all muscles during a twitch, if the conclusion is correct that in a variety of muscles activation is a function of tension as well as of time.

The presence of a "series elastic component" in muscle is indeed very strongly implied by a variety of experiments (Wilkie, 1956), although its structural whereabouts and chemical nature are unknown. From a theoretical viewpoint, this component is of great significance, since the mechanical properties of muscle, as currently visualized, depend on it. It is supposed to provide a "buffering" action in muscle against the consequences of rapid changes in physical state. It is possible, however, that this elastic component is not a structural entity but is part of the contractile machinery left in the elastic ("resting") state when other portions are already activated. If the contractile system is not activated at the same instant along the whole length of the fiber, the elastic, not yet activated, portion can indeed fulfill the buffering function. A comparative study of cross-striated and smooth muscle, with respect to the series elastic component, may explore this question.

The "length-tension" and "load-shortening velocity" relations occupy a central point in the study of muscle physiology, and have yielded significant conclusions since their first discovery. From the load-shortening velocity relation, Hill's classic equation was derived: $(P + a)$ $(V + b) =$

interpretations of the obtained data, even if one assumes that the methods of analysis of ~ph are accurate enough for the demonstration of a fraction of micromolar change in ~ph content during a single muscle twitch and also that the control and experimental samples are identical before the experiment. Quick cooling, preceding freezing, is known to activate muscles and recent experiments (Csapo, unpublished) indicate that muscle, whether electrically or pharmacologically stimulated or "resting", may be equally and fully activated when frozen. The lack of action potentials is no evidence against myoplasmic activity since both normally polarized and depolarized muscles are equally affected by quick freezing. Nor is the lack of mechanical response a proof of rest, because activation occurs several milliseconds earlier than the mechanical response; if freezing quickly follows cooling, the two subsequent processes, activation and mechanical response may be interrupted. One must insist, therefore, that a state of rest be proved rather than taken for granted. A great number of methods are known by which muscle can be effectively activated. The significance and also the necessity of learning about how to keep muscles at rest is now being realized. In our experiments, it was possible to suspend the effect of quick freezing only by pretreating the muscle with Na Versene.

These considerations about energetics suggest, as did those concerning structure and contractile proteins, that the molecular structure of the contractile machinery and the principles of its function may be similar in variety of muscle tissues. We may justly expect similarities among them in mechanical properties.

VI. MECHANICAL PROPERTIES

Resting muscle is plastic and freely extensible. During excitation it passes into a new physical state; it becomes hard, develops tension, lifts load, and resists stretching. This is the "active state", first described by Gasser and Hill (1924), and characteristic of all muscles. But while in the frog sartorius (at 0°C.) the "active state" manifests itself 3 msec. after the beginning of stimulation and full activity is reached after about 20 msec.; in uterine muscle, activation proceeds very slowly (even at 37°C.), reaching maximum about 4 sec. after stimulation (Csapo and Goodall, 1954). Since the mechanical latency period in the uterus is about 200 msec., it was concluded from these observations that activation in this muscle is a function of tension as well as of time. Goodall (1955) studied in the uterus the rate of the redevelopment of

VII. SOME TECHNICAL CONSIDERATIONS

It is not altogether clear to many students of smooth muscle that it is significant whether the mechanical response of intact muscle is studied isotonically or isometrically. In the former case, the muscle shortens and lifts load; the work done = load × distance. For each muscle, there is only one specific load which when lifted yields maximum work, and this is approximately one-half of the maximum load which the muscle can sustain but not lift. This maximum load is equal

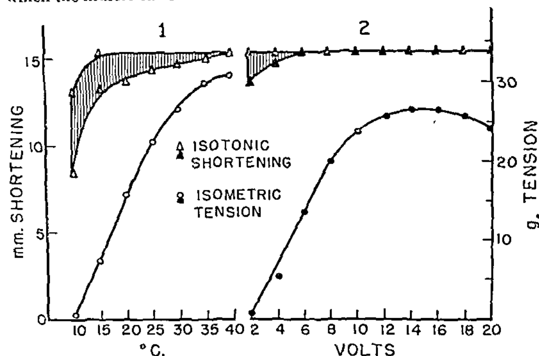


FIG. 10. Isometric tension and isotonic shortening of the same uterine segment as a function of temperature and the strength of electrical stimulus (Csapo, 1954a). Hatched areas indicate variation of individual samples. Note that shortening of the unloaded uterus is practically maximal when tension is reduced to about 5%.

to the isometric force which develops in a tetanus without external shortening.

In a series of experiments (Csapo 1954a), isotonic shortening was compared with isometric tension of the same muscle, by varying the strength of stimulation and temperature. When tension was recorded, increasing field strength and temperature resulted in a gradual increment of tension. An unloaded muscle shortens maximally at the threshold stimulus and temperature (about 10°C.). This means that if there is no resistance against which the muscle is to work, shortening is maximal even though only a fraction of the contractile system is

$(P_o + a)\, b$ (Wilkie, 1956). Considering the similarities between cross-striated and smooth muscle previously pointed out, it is not surprising that the uterus obeys both relations very accurately (Csapo and Goodall, 1954). This similarity in behavior between the two types of muscle is indeed important because it suggests that whatever is the underlying mechanism of contraction, it is the same in a variety of muscles.

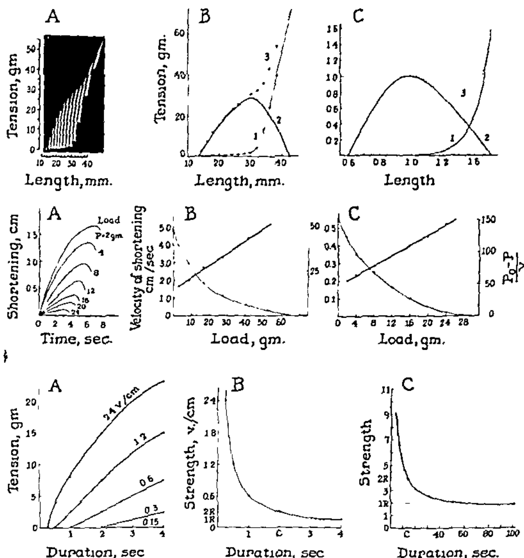


Fig. 9 The length-tension, load-shortening velocity, and strength-duration relations of the frog sartorius and the rabbit uterus (after Hill, 1953; Rushton, 1930; Csapo and Goodall, 1954). The first picture in each row is an original record from an experiment with the uterus; the second is a plot from the first record to be compared with the third in each row, illustrating the same experiment with the frog sartorius. Note the general similarities between the frog sartorius and the rabbit uterus, in each of the three relations.

is an expensive and time-consuming affair since elaborate synthetic processes are involved which may only be justified in special cases like those of pregnancy and parturition. It is certainly simpler to shift ions around so as to alter the threshold, conductivity, and probably the coupling process. These considerations are mentioned to point out the possibility that the excitation process may be a bottleneck in the physiological function and regulation of different muscles.

Excitable tissues are all alike in that the intracellular [K] and [Na] are reversed with respect to their surrounding medium and that the inside of the cell is electrically negative with respect to the outside, giving rise to a potential difference across the excitable membrane (Hodgkin, 1951).

The "threshold" relation informs us (Jernerick and Gerard, 1953) that effective excitation in intact muscle occurs when the membrane potential (96 mv. in the frog sartorius) is suddenly decreased to a "critical" value (52–56 mv. in the frog sartorius). The difference between membrane "resting" and "critical" potential is called "excess" or "safety" potential. The stimulus needed for effective excitation depends on the magnitude of the excess potential being zero when the muscle is depolarized to the critical level.

It is implied in the threshold relation that the contractile system in muscle is so balanced that change-over from rest to activity occurs automatically unless an excess or safety potential prevents the transformation. From this relation, one also would expect that muscles kept below the critical potential would stay in contracture. The first muscle, however, which disappoints the observer in these expectations is the frog sartorius, the very one on which the threshold relation was first established. If the frog sartorius is depolarized to the critical potential, recording of isometric tension reveals no appreciable spontaneous activity. If the sartorius is suddenly and excessively depolarized, contracture does occur but it is only transient and subsides in a few seconds. Why the frog sartorius does not develop effective spontaneous activity at the critical potential and why contracture is so short-lived when the muscle is depolarized below the critical potential are puzzling questions. This situation is more puzzling because it has been shown (Csapo, 1956) that the smooth muscle of the uterus obeys the threshold relation fully in exhibiting maximum spontaneous activity at the critical potential and sustained contracture below it (Fig. 11).

A possible explanation of this difference in behavior between cross-

activated (about 5%). Isotonic recording with little or no load, as commonly and uncritically used, is therefore misleading, and is only justified in a few special cases. Since most pharmacological studies on smooth muscle have been carried out with unloaded or slightly loaded levers, their results are often meaningless.

Isometric recording is not the only necessary experimental practice. In a quantitative study of muscle function, special attention should also be paid to the following factors in experiments with mammalian smooth muscles: the use of mammalian Krebs solution, oxygenation with 95% O_2 + 5% CO_2, control of pH and temperature, and quick transfer of the tissue from the living animal to the perfusion Krebs solution. Smooth muscle can be stored, but only under carefully controlled conditions, so as to maintain the normal ionic gradients.

It is often necessary to record the work done or the tension developed by smooth muscles in the living animal. Studies of this type (Schofield, 1954; Bengtsson, 1957) on the rabbit uterus have been carried to a considerable state of perfection. If the anesthesia and the body temperature of the animal and of the recording chamber are well controlled, no difference can be detected between the performance of the excised and the *in situ* uterus. These experiments have confirmed phenomenologically the characteristic effects of estrogen and progesterone on the myometrium, described previously on the excised uterus (Csapo, 1955, 1956).

VIII. EXCITABILITY

The importance of studying the excitability of smooth muscles is now fully realized. These studies allow us to propose certain generalizations about excitability, to specify some unique features of excitation in smooth muscle contrasted to that of cross-striated muscle, to investigate the mechanism of regulation in terms of hormone and drug effects on excitability, and even to make useful suggestions to the practically minded who are concerned with disease and treatment. Excitation is the first in a series of processes which if effective can yield muscular work. The others are the coupling process and the mechanical response. If the first process fails, the others will not take place although potentially they may be set to occur. It is enough then to suspend excitation in order to eliminate muscle function and there is evidence to suspect that nature takes advantage of this possibility when needed. As pointed out previously, the working capacity of the uterus depends upon the action of estrogen building up the final contractile system, but this

striated and uterine muscle is offered by some of our recent experiments. Turtle muscle behaves as if a critical fraction of its Ca would be lost as a function of the extent of sustained depolarization, elicited by excess potassium. That it is the escape of calcium which results in inexcitability at a $[K]_o$ of for example, 24–28 mM per liter is indicated by the observation that the muscle response to electrical stimulation returns under these conditions as a function of the $[Ca]_o$. The uterus unlike frog and turtle cross-striated muscles retains its maximum response to a strong longitudinal field, even when completely depolarized by excess potassium.

It appears that depolarized cross-striated muscle becomes extremely sensitive to Ca-deficiency which causes it to become "uncoupled." Treatment with excess calcium "recouples" the activation chain and the muscle response to electrical stimulation returns.

The membrane potential of the frog sartorius is not only high (96 mv), but is also stable. In smooth muscles, on the contrary, it is not only low but its scatter is very great. In the pregnant uterus Woodbury and McIntyre (1954) reported a scatter as large as 22 to 66 mv., and in the taenia coli Bulbring (1954) recorded values between 22 to 78 mv. This scatter may be due in part to damage of the small cell (5 to 10 μ diameter) during impalement as suggested by Bülbring. But it may also be an expression (Csapo, 1956) of a continuous rhythmic membrane activity.

Our experiments (Goto and Csapo, 1958) showed that the uterus, not acted upon effectively by the ovarian steroids, has a low membrane potential of about 30 mv. A few days of estrogen treatment results in a rise in potential to a value of about 45 mv. Such a muscle has good excitability, conduction, and pharmacological reactivity. Progesterone treatment (in addition to estrogen) leads to a further increment in membrane potential up to 55 to 60 mv., a loss of conduction and pharmacological reactivity.

It is of interest that the uterus dominated by progesterone binds calcium more effectively than that dominated by estrogen (Coutinho and Csapo, 1958). This difference in Ca-binding could explain the functional properties of the uterus under these contrasting endocrine conditions. The elegant experiment of Bulbring *et al.* (1956) should also be recalled demonstrating that the membrane potential of the Ca-deficient frog muscle becomes labile, and its value scatters like that of smooth muscle.

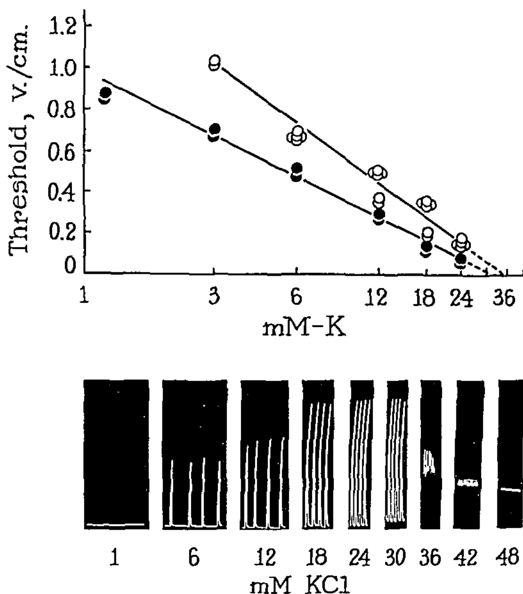


FIG. 11 The threshold relation and the effect of depolarization on the spontaneous mechanical activity of the rabbit uterus (Csapo, 1956). The upper graph illustrates that the strength of stimulation required to elicit a threshold response decreases as

mM per liter The lower graph illustrates that the frequency and magnitude of the rythmic isometric contractions increase with gradual depolarization, elicited by increasing the $[K]_o$ Note that hyperpolarization ($[K]_o = 1$ mM per liter abolishes spontaneous activity, that tension is maximal at $[K]_o = 30$ mM per liter) and that sustained contracture develops if the muscle is depolarized below the "critical potential," i e at $[K]_o > 30$ mM per liter

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It appears that the physical state of muscle calcium is influenced by regulation. We observed (Coutinho and Csapo, 1958) that the Ca-deficient uterus, whose tetanus is barely measureable, develops maximum tension if treated by oxytocin. This suggests that oxytocin might act as "Ca-carrier" (Figs. 12 and 13).

A puzzling question is how spontaneous activity originates in smooth

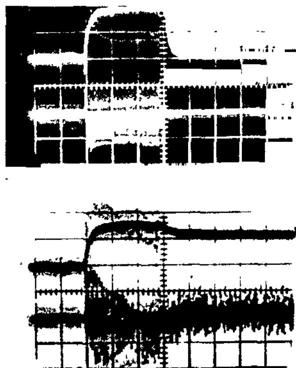


FIG. 12. Delayed relaxation and "repetitive firing" of the Ca-deficient frog toe muscle *(Kernan and Csapo, 1957). The upper record shows a train of action potentials in normal muscle giving rise to a 1-sec. tetanus.* Note that electrical and mechanical activity suddenly ceases with the last normal action potential elicited by electrical stimulation The lower record shows the same muscle 10 minutes after treatment with Ca-deficient Ringer Note that after the last normal action potential "repetitive firing" continues automatically without stimulation, and that tension is maintained

muscles. The correlation between membrane and mechanical activity is well established by Bulbring (1956). As for rhythmic membrane activity all we know is that such activity is greatly dependent on temperature and O_2, and that muscles possessing such activity have a low [CP], which has been correlated with low values of excess potential (Ling, 1952) and with membrane lability. A possible working hypo-

thesis which explains these observations is that spontaneous activity involves a cyclic release and readsorption of calcium. Agents that influence the forces by which calcium is held in muscle or interfere with its readsorption influence spontaneous activity. The relationship mentioned previously between depolarization, calcium release, and excitability is of special importance here.

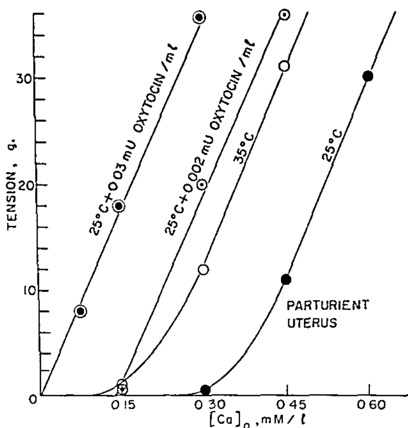


FIG. 13. The effect of temperature, $[Ca]_o$, and oxytocin on the tension developed by the Ca-deficient uterus (Coutinho and Csapo, 1958). Note that tension of the Ca-deficient muscle (after repeated washing in Ca-free Krebs), at 25° C and at a $[Ca]_o$ = 0.30 mM per liter, is just measurable. It increases, however, to maximal at an oxytocin concentration of 0.03 mU per milliliter. The correlation between tension and temperature, $[Ca]_o$, and oxytocin provides an accurate method for the quantitative estimation of oxytocin concentration.

Other evidence of membrane lability is the demonstration (Kernan and Csapo, 1957) that the frog toe muscle in Ca-deficient Ringer increases its time of relaxation fivefold, a retarded "deactivation" of the contractile system which coincides with "repetitive firing" of the membrane after the cessation of stimulation. Prolonged relaxation is a characteristic property of intact uterine muscle, and the fact that the

frog sartorius can assume similar properties when slightly Ca-deficient indicates that some of the striking and pronounced differences between one muscle and another may find their explanation in the concentration and physical state of muscle (membrane) calcium.

The most striking property of the excitable membrane is its built-in mechanism for the generation and conduction of propagated activity, in the form of an "action potential." It was already known to Bozler (1938) that the uterus propagates membrane activity. It is comforting to learn from the recent observations of Woodbury and McIntyre (1956), using the microelectrode, that action potentials with overshot can be recorded from uterine muscle cells. The significance of the microelectrode recording is the demonstration that a mechanism operates in uterine muscle during membrane activity similar to that of other excitable tissues.

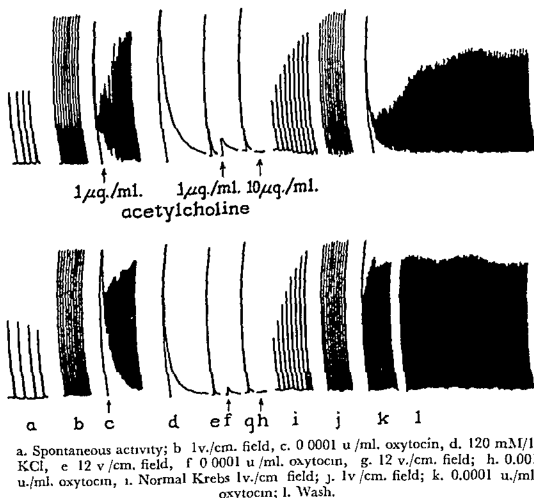
IX. PHARMACOLOGICAL STIMULATION

From these experiments and from earlier ones on isolated AM it was anticipated that pharmacological stimulation involves intact membrane function. In a series of experiments, Csapo (1947, unpublished) found that estrogen, progesterone, oxytocin, acetylcholine, histamine, and adrenalin do not affect the characteristic viscosity change and thread contraction of isolated AM, initiated by ATP. In another set of experiments, (Csapo, 1954b, 1957) the question whether depolarization eliminates pharmacological stimulation was studied.

Intact uterine strips were tetanized and maximum tension recorded. Then the maximum response to pharmacological agents was observed, *including in the series: oxytocin, acetylcholine, histamine, and serotonin.* In the next step the uterus was completely depolarized by immersing it in a mammalian Krebs solution with excess potassium (K = 120 mM per liter—substituted for Na). By this treatment, both the K and Na gradients were eliminated. When placed in this modified Krebs solution, the uterus developed maximum contracture tension, which however gradually subsided, the uterus resuming its original resting length after some 15 minutes. The uterus in this condition is a *resting muscle, with very little if any membrane potential. Rest is* evidenced by the length of the muscle and by the fact that it can be repeatedly activated in a longitudinal a c. field (Csapo, 1954b) and d.c. field (Mashima and Csapo, 1957) of sufficient strength, resulting in maximum tension. The transverse field, a.c. or d.c., is relatively in-

effective. This demonstration is also proof of the intact state of the contractile system and shows that activation can be achieved by current flow in the absence of the membrane potential.

The point of interest here is the finding that the uterus in this depolarized condition does not exhibit the characteristic pharmacological effects, in spite of the fact that its contractile system retains maximum



a. Spontaneous activity; b 1v./cm. field, c. 0 0001 u /ml. oxytocin, d. 120 mM/l. KCl, e 12 v /cm. field, f 0 0001 u /ml. oxytocin, g. 12 v./cm. field; h. 0.001 u./ml. oxytocin, i. Normal Krebs 1v./cm field; j. 1v /cm. field; k. 0.0001 u./ml. oxytocin; l. Wash.

FIG. 14 The maximum "myoplasmic excitability" and the lack of pharmacological reactivity of the K-depolarized uterus (Csapo, 1954b, 1957). In a modified 120 mM per liter K and 35 mM per liter Na Krebs solution, the uterus develops a transient contracture followed by rest In the absence of potassium and sodium gradients and membrane potential, maximum tension develops repeatedly in a longitudinal a c. field of sufficient strength (12 v. per centimeter). Note the lack of pharmacological reactivity in spite of maximum contractile capacity, and also the complete recovery on repolarization and return of normal excitability (1 v. per centimeter) and pharmacological reactivity.

contractile capacity. A contracture of less than 10% can be elicited by the administration of these drugs, but a second application is without effect even if the concentration of the substance is increased 10–100 times. Our conclusion is that "muscle stimulants" do not stimulate the contractile system at all, but regulate membrane function so as to make the intrinsic stimulatory system more effective. Characteristic drug response requires intact membrane function. Experiments yielding different results from the one just described were reported by Evans and Schild (1957), who state that depolarized smooth muscle responds to acetylcholine. In these experiments, isotonic recording was employed. Since this technique does not describe the extent of activation, no quantitative conclusions can be drawn from these studies.

These observations and the experience that the isolated contractile system is only sensitive to temperature and charges (Szent-Gyorgyi, 1953) suggest that "regulatory influences" have to be translated at the membrane into terms of charges if an effect is expected at the level of the final contractile system. Charge displacement may be an ancient method of regulation, discovered at a time when there were no other means of regulating the primitive function of the first protein. In the course of evolution, as cells, organs, and whole animals developed and as they became increasingly complex, regulation also had to become specific and localized. A variety of compounds with adequate specificity had to be created to impose regulation upon such a complexity. Thus perhaps membranes learned how to "translate" while the cytoplasms remained faithful to the old regime.

X. EXCITABILITY OF THE POTASSIUM-DEPOLARIZED UTERUS

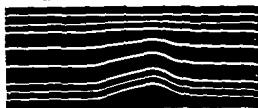
In his "Facts and Theories About Muscle" Wilkie (1954) points out that "few of us are strong-minded enough to follow Bateson's advice 'to treasure our exceptions'." The following experiments illustrate the usefulness of this moral. It was reported (Csapo, 1954b) that the smooth muscle of the rabbit uterus can be completely depolarized by immersing it in a high potassium (120 mM per liter) and low sodium (35 mM per liter) Krebs solution, without loss of electrical excitability and contractility. This condition prevails for more than 60 minutes, during which time the muscle can be repeatedly tetanized by strong longitudinal current. The electric field applied in the transverse direction is little, if at all, effective. When the uterus is repolarized in a regular Krebs solution, all properties of the intact myometrium return. In this

condition the potassium and sodium gradients across the membrane are eliminated and there is no appreciable membrane potential. This exceptional behavior of the uterus strongly suggested, in spite of contrary views, that depolarization and activation are not directly linked to one another but that an additional step in the activation process is involved, subsequent to depolarization. This step seems to be dissipated by partial and sustained depolarization in frog muscle but is maintained in the uterus, completely depolarized. Since longitudinal but not transverse

Turtle, retracter penis. 20° C. Load: 2 g.



Ringer L 1 v./cm. a.c.



20 mM/l K Ringer
L 4 v./cm. d.c.



Ringer L 1 v./cm. a.c.

FIG. 15. Illustration of a new technique for recording shortening in different portions along the length of a muscle (Mashima and Csapo, 1957). The retractor penis of the turtle is marked off into 7 segments along its length with a fluorescent dye (RCA→33-Z-607). The movement of the illuminated marks is recorded by a constant speed motion picture camera. The record obtained is shown here. Graphs 1 and 2 are normal tetani before the experiment and after recovery, respectively. Note that shortening is uniform along the whole length. Graph 2 illustrates shortening in a 20 mM per liter potassium turtle-Ringer solution and in a 4 v. per centimeter d.c. field. Note that shortening involves the whole length, except the extreme anodal end, and that shortening is the greatest in the middle portion, and at the extreme cathodal end.

applied current effectively and fully activates the depolarized resting uterus, it was thought that internal currents complete the "priming" effect of depolarization in the activation of the contractile machinery.

XI. EXCITATION-CONTRACTION COUPLING

The coupling process is an old problem of muscle physiology. The current position has been recently expressed by A. F. Huxley (1956) stating that the essential thing in activation is the change in the potential difference across the membrane itself, and currents flowing inside the muscle fibers are ineffective.

Our own observations on the other hand suggest (Csapo and Suzuki, 1957; Mashima and Csapo, 1957; Csapo, 1957; Csapo and Suzuki, 1958; Sakai and Csapo, 1958) that activation is a joint contribution of depolarization and current flow. Unless the participation of ionic current in the activation process is definitely ruled out by direct evidence, it is justified to consider its effectiveness (Fig. 15).

Since it is difficult, if at all possible, to provide experimental conditions whereby the muscle becomes depolarized with the complete absence of current flow, in recent experiments we have taken the opposite approach. Using the intracellular microelectrode technique, combined with a method (Mashima and Csapo, 1957) which accurately records shortening along the length of the muscle, we have shown (Sakai and Csapo, 1958) that K-depolarized, nonpropagating turtle muscle can shorten in a longitudinal d.c. field at portions where the membrane potential remains unchanged or increases slightly during stimulation (Fig. 16).

When the longitudinal d.c. is abruptly applied the muscle shortens along its entire length (except at the extreme anodal end). If the current is gradually increased, however, the cathodal end hardly shortens, whereas shortening at other portions is unchanged. Thus the rate of the membrane potential change is critical for the effect at the cathode whereas shortening at the middle portion of the muscle is dependent only on the applied current and independent of the membrane potential change (Fig. 17).

Measurements of the membrane potential change with microelectrodes rule out the possibility that the middle and anodal portions of the nonpropagating muscle become depolarized in the d.c. field. Shortening in these portions then is evidence for the triggering effect of internal currents. Furthermore if the effect of the longitudinal field

were mediated by depolarization (due to distortion of the applied current by irregularities in the fiber), the transverse d.c. field should be more effective than the longitudinal. In fact just the opposite is true as shown by our experiment on the turtle muscle at $[K]_o = 20$ mM per liter.

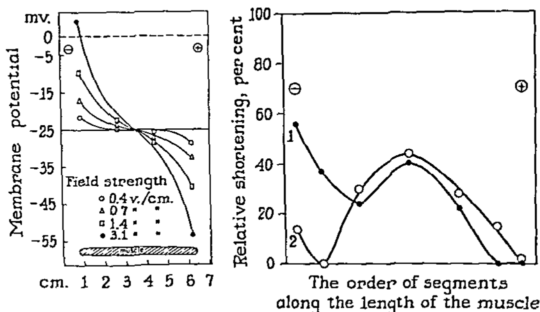
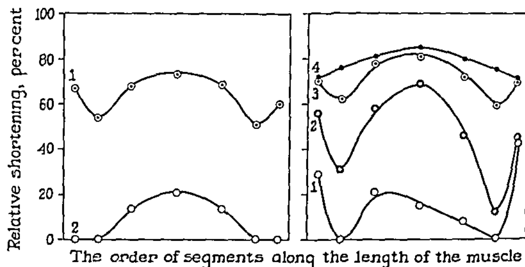


FIG. 16. Left: The change in membrane potential along the length of the K-
depc
d.c.
mete
at the anodal portion of the muscle (Sakai and Csapo, 1958).
Right: Shortening of the K-depolarized (nonpropagating) turtle muscle along its length elicited by longitudinal d.c. The $[K]_o = 20$ mM per liter; the strength of stimulation: 6 v. per centimeter; load = 3 g. Note that the middle and anodal half of the muscle does shorten. Note also that if the stimulating current is slowly (Curve 2) rather than abruptly (Curve 1) applied, the cathodal effect is greatly reduced (Mashima and Csapo, 1957).

We believe that depolarization is an essential first step in the series of events leading to myoplasmic activity. They are not linked directly, however, but are connected by at least one intermediate step in which calcium is involved. We have succeeded in "uncoupling" the turtle muscle temporarily at a membrane potential above the "critical" value, by rendering it Ca-deficient or by recovering it from excess potassium treatment at low temperature (see also Csapo and Wilkie, 1956). Excess

calcium in both cases shortens the time during which the muscle is "uncoupled."

It appears that depolarization "primes" the muscle for activity by calcium release, and that this ion may be effectively carried by current, thereby contributing to the coupling process. The sensitivity of the muscle to current seems to depend on the extent of this priming effect



ably. Right: Frog toe muscle, $[K]_o$ = 16 mM. Stimulus: longitudinal a c. = 3, 6, 12 and 16 v./cm respectively (Mashima and Csapo, 1957).

of depolarization. If, on the other hand, depolarization is maintained, allowing ample time during which calcium is free to move outward with the gradient away from the active sites, the sensitivity of the muscle to current decreases.

XII. A LINK BETWEEN INTACT MUSCLE AND ITS MODELS.

The experiments just described opened up the possibility of studying depolarized muscle as a "link" between intact muscle and its "models." When Szent-Gyorgyi first claimed that muscular contraction is an interaction between AM, ATP, and ions, many objections were raised in different quarters against his conclusion, on the ground that his greatly simplified muscle preparations had no resemblance in their contractile properties to those of intact muscle. How isolated AM or

glycerol-treated muscle should have behaved, of course, nobody knew since no one has ever seen contracting AM in intact muscle, free of the regulating influences of this complex biological system. One suspects that the regulatory influences of the fiber membrane would mask the genuine properties of the myoplasm, but if one could reduce this regulatory influence by temporarily suspending it in the intact muscle, then

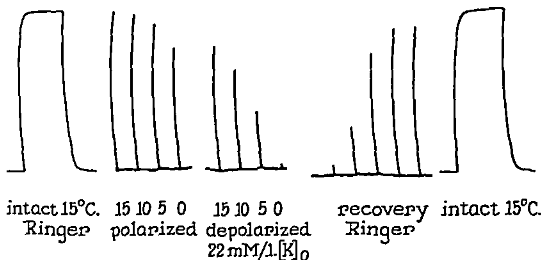


FIG. 18 The temperature dependence of the intact and K-depolarized frog sartorius (Csapo, 1957). Note that tension is more sensitive to temperature in the K-depolarized than in the intact polarized muscle.

one can expect that this simplified muscle and the "models" would exhibit some similar properties (Fig. 18).

Intact frog muscle, somewhat simplified by K-depolarization, can be repeatedly tetanized in a strong electric field. Contrary to the behavior of intact polarized muscle, which is insensitive to changes in pH and temperature (Csapo and Wilkie, 1956), the depolarized sartorius is greatly dependent on pH and temperature (Csapo, 1957). Tunic (personal communication) finds a Q_{10} in the Hayashi model higher than 2.5, and Briggs and Portzehl (1957) reported that tension of the glycerinated psoas is abolished if the pH is lowered from 7.2 to 6.2. The K-depolarized intact sartorius exhibits similar properties, while it becomes again insensitive to temperature and pH when repolarized (Csapo, 1957) (Fig. 19).

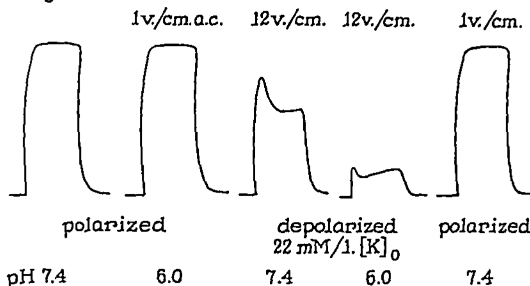


FIG. 19. The pH dependence of the intact and K-depolarized frog sartorius (Csapo, 1957). Note that intact muscle is insensitive to changes of the pH of the perfusing Ringers solution, whereas tension of the K-depolarized muscle is greatly reduced if the pH is lowered from 7.4 to 6.0.

XIII. CONCLUDING REMARKS

For those who have never worked with smooth muscle or who used it only to test pharmacological effects, the term "headache" muscle may sound appropriate. But to those who appreciate the observations that from these muscles a contractile system was isolated greatly similar to that of skeletal muscle; that smooth muscle exhibits many of the classic properties of muscle in excitability and mechanical response such as length-tension, strength-duration, load-shortening velocity, and threshold relations; and that it obeys Hill's equation, this term is no longer acceptable. Smooth muscle, however, may still remain a headache muscle for those who intend to make generalizations about structure and function of muscle on experimental grounds restricted to the frog sartorius or the rabbit psoas.

REFERENCES

Bengtsson, L. P. (1957). *Am. J. Obstet. Gynecol.* **74**, 484
Blasius, R., and Schuck, J. (1955). *Klin Wochschr.* **33**, 276.
Born, G. V. R. (1956) *J. Physiol.* **131**, 704
Bozler, E. (1938). *Am. J Physiol.* **122**, 614.
Briggs, F. N., and Portzehl, H. (1957) *Biochem. et Biophys. Acta* **24**, 488.
Bulbring, E (1954). *J Physiol.* **125**, 302
Bulbring, E. (1956). *20th Intern Congr. Physiol. Sci. Brussels.*
Bulbring, E , Holman, M., and Lullman, H (1956). *J. Physiol.* **133**, 101.

Coutinho, E., and Csapo (1958) *Biol. Bull.* **115**, 334.
Cretius, K. (1957). *Gynaecologia* **143**, 192.
Cretius, K. (1958). *Fortschritte* der *Geburtshilfe* und *Gynakologie*, **7**, 29.
Csapo, A. (1948). *Nature* **162**, 218.
Csapo, A. (1949). *Acta Physiol. Scand.* **19**, 100.
Csapo, A. (1950). *Am. J. Physiol.* **162**, 406.
Csapo, A. (1954a). *Am. J. Physiol.* **177**, 348.
Csapo, A. (1954b). *Nature* **173**, 1019.
Csapo, A. (1955). *In* "Modern Trends in Obstetrics and Gynaecology" (K. Bowes ed.), Second Series, Chapter 2. Butterworths, London.
Csapo, A. (1956). *J. Physiol.* **133**, 145.
Csapo, A. (1957). *Nat. Acad. Sci. "Autumn Meeting New York."* 5.
Csapo, A., and Corner, G. W. (1953). *Science* **117**, 162.
Csapo, A., and Gergely, J. (1950). *Nature* **166**, 1078.
Csapo, A., and Goodall, M. C. (1954). *J. Physiol.* **126**, 384.
Csapo, A., and Hermann, H. (1951). *Am. J. Physiol.* **165**, 701.
Csapo, A., and Suzuki, T. (1957). *Proc. Natl. Acad. Sci. U.S.* **43**, 278.
Csapo, A., and Wilkie, D. R. (1956). *J. Physiol.* **134**, 497.
Evans, D. H. L., and Schild, H. O. (1957). *J. Physiol.* **136**, 36.
Fleckenstein, A., Janke, J., Davis, R. E., and Krebs, H. A. (1954). *Nature* **174**, 1051.
Gasser, H. S., and Hill, A. V. (1924). *Proc. Roy. Soc.* **B96**, 398.
Gergely, J. (1953). *J. Biol. Chem.* **200**, 543.
Gergely, J., and Kohler, H. (1957). *Conf. Chem. Muscular Contraction Tokyo* **9**, 66.
Gergely, J., Gouvea, M. A., and Karibian, D. (1955). *J. Biol. Chem.* **212**, 165.
Goodall, M. C. (1955). *J. Cellular Comp. Physiol.* **46**, 356.
Goto, M., and Csapo, A. (1958). *Biol. Bull.* **155**, 335.
Hayashi, T., Rosenbluth, R., Satir, P., and Vozick, M. (1956). *Biol. Bull.* **111**, 290.
Hill, A. V. (1953). *Proc. Roy. Soc.* **141**, 104.
Hill, A. V. (1956). *Brit. Med. Bull.* **12**, 174.
Hippel, P. H., Gellert, M. F., and Morales, M. (1957). *Conf. Chem. Muscular Contraction Tokyo* p. .
Hodge, A. J., Huxley, H. E., and Spiro, O. (1954). *J. Exptl. Med.* **99**, 201.
Hodgkin, A. L. (1951). *Biol. Revs. Cambridge Phil. Soc.* **26**, 339.
Huxley, A. F. (1956). *Brit. Med. Bull.* **12**, 167.
Huxley, A. F. (1957). *Progr. in Biophys. and Biophys. Chem.* **7**, 255.
Huxley, H. E. (1956). *Brit. Med. Bull.* **12**, 171.
Jernerick, H. P., and Gerard, R. W. (1953). *J. Cellular Comp. Physiol.* **42**, 79.
Kawaguti, S., and Ikemoto, N (1957). *Biol. J. Okayama Univ.* **3**, 196.
Kernan, R. P., and Csapo, A. (1957). *Biol. Bull.* **113**, 329.
Kominz, D. R., and Saad, F. (1956). *Federation Proc.* **15**, 112.
Laki, K. (1957). *J Cellular Comp Physiol.* **49**, 249.
Lange, G. (1955). *Biochem. Z.* **326**, 172.
Ling, G. N. (1952). *In* "Phosphorus Metabolism" (W. D. McElroy and B. Glass, eds.), Vol. 2, p. 748. Johns Hopkins Press, Baltimore, Maryland.
Lohmann, K. (1934). *Biochem. Z.* **271**, 264.
Lundsgaard, E. (1930). *Biochem. Z.* **227**, 51.
Lundsgaard, E. (1934). *Biochem. Z.* **269**, 308.
Mark, J. S T. (1956) *Anat. Record* **125**, 473.
Mashima, H., and Csapo, A. (1957). *Biol. Bull.* **113**, 349.
Menkes, J., and Csapo, A. (1952). *Endocrinology* **50**, 37.
Mommaerts, W. F. H. M. (1954). *Nature* **174**, 1038.
Munch-Petersen, A. (1953). *Acta Physiol. Scand.* **29**, 202.

Needham, D. M. (1956). *Brit. Med. Bull.* **12**, 194.
Needham, D. M., and Cawkwell, J. M. (1957). *Biochem. J.* **65**, 540.
Porter, K. R., and Palade, G. E. (1957). *J. Biophys. Biochem. Cytol.* **3**, 269.
Rushton, W. A. H. (1930). *J. Physiol.* **70**, 317.
Sakai, T., and Csapo, A. (1958). *Biol. Bull.* **115**, 341.
Schofield, B. M. (1954). *Endocrinology* **44**, 142.
Sjostrand, F. S., and Andersson-Cedergren, E. (1957). *15th Ann. Meeting Electron Microscope Soc. Am. Cambridge Mass.* p. .
Snellman, O., and Tenow, M. (1954). *Biochem. et Biophys. Acta* **13**, 199.
Szent-Gyorgyi, A. (1953). "Chemical Physiology of Contraction in Body and Heart Muscle." Academic Press, New York.
Szent-Gyorgyi, A. (1958). *Science*, **128**, 699.
Tonomura, Y., and Sasaki, A. T. (1957). *Enzymologia* **18**, 111.
Tonomura, Y., Yugi, K., and Matsumiya, H. (1957). *Arch. Biochem. et Biophys.* **64**, 466.
Weinstein, H. J., and Ralph, P. H. (1951). *Proc. Roy. Soc. Exptl. Biol. Med.* **78**, 614.
Wilkie, D. R. (1954). *Progr. in Biophys* and *Biophys. Chem.* **4**, 288.
Wilkie, D. R. (1956). *Brit. Med. Bull.* **12**, 177.
Woodbury, J. W., and McIntyre, D. M. (1954). *Am. J. Physiol.* **177**, 355.
Woodbury, J. W., and McIntyre, D. M. (1956). *Am. J. Physiol.* **187**, 338.

CHAPTER IX

Structure and Function of the Contractile Apparatus in the Muscles of Invertebrate Animals

JEAN HANSON AND J. LOWY

I. INTRODUCTION

Contractility is a universal property of living cells. Only by comparing different contractile systems in considerable detail will it be possible to decide what molecular mechanisms are common to all of them. Here we are concerned with the muscles of invertebrate animals, which are very important from this point of view. In invertebrates, every type of muscle is represented, and some of them are so remarkably specialized that unique mechanisms have been proposed to explain how they work.

It should not be forgotten that the way a muscle functions in a living

animal depends not only on the properties of its contractile elements but also on how they are brought into action. Other important controlling factors include passive muscle elements such as connective tissues. Here attention will be concentrated on the intrinsic properties of the contractile material, its construction, its mechanical performance, and its constituent proteins. But as far as possible, these features will be related to the function which the particular muscle carries out in life.

II. SURVEY OF THE ANIMAL KINGDOM

A. Introduction

A brief survey of the distribution of the different types of muscle fibers in the animal kingdom, and of the purposes for which they are used, will serve to bring out any obvious relationships between structure and function, and will also prepare the way for the more detailed discussions of physiology, structure, and biochemistry which follow. Few comprehensive reviews of this kind have ever been attempted (Prenant, 1912; Hoyle, 1957), and it is difficult to collect the information which is needed, for much of it is contained in papers dealing with other aspects of zoology. The references given here do not aim to be complete; they have been critically selected, rejecting many unsupported statements, and they direct attention to key papers, to the recent postwar literature, and sometimes to interesting early observations.

B. Provisional Classification of Muscles on the Basis of Fiber Structure

A classification of muscles on the basis of the structure of their fibers is at present bound to be unsatisfactory, for recent results obtained by electron microscopy have shown the limitations the light microscope imposes on the recognition of significant structural detail, and as yet very little is known about the submicroscopic morphology of smooth muscles. The provisional scheme adopted here recognizes four classes, one comprising the striated muscles, and the other three containing different kinds of unstriated (smooth) muscles.

1. *Striated Muscles*

The contractile elements (Figs. 5, 6, and 19) are axially differentiated into an alternating sequence of optically dense, birefringent A bands,

and less dense, less birefringent I bands. The banding is caused by the way in which submicroscopic protein filaments of two kinds are arranged in the structure (see Volume I, Chapter VII). The electron microscope has not yet discovered any muscle with an axial periodicity of this kind that cannot be resolved by light microscopy.

2. *Helical Smooth Muscles*

These are muscles with myofibrils (i.e. bundles of filaments) which follow a helical course in the fiber (Figs. 1, 2, and 3). Such fibers show "double-oblique striation" in the light microscope, i.e. a diamond lattice appearance due to the visibility of both aspects of the helix when the microscope is appropriately focused. Not all muscles with "double-oblique striation" actually possess helical fibrils; some show the diamond pattern only when they are excessively contracted, and in these cases the lines of the lattice probably represent contraction bands (Fig. 9).

3. *Paramyosin Smooth Muscles*

These are smooth muscles (Figs. 10 and 13–17) whose fibers contain longitudinal filaments of large size (sometimes more than 0.1 μ in diameter) and complex structure, which appear to be composed of ribbon-shaped "paramyosin" elements. The latter have a characteristic structure which is visible in the electron microscope and readily detected in low-angle X-ray diffraction patterns. The name "paramyosin" refers to this structure, which is very probably due to the protein tropomyosin.

4. *Classic Smooth Muscles*

These are muscles which are smooth but are neither of the "helical" nor of the "paramyosin" type (Fig. 12). This category no doubt contains muscles of several different kinds, and some which are at present included in it may belong to the paramyosin smooth muscle category but have not yet been adequately studied.

C. Distribution of the Main Types of Muscle Fibers

1. *Striated Muscles*

Striated muscles are present in nearly all kinds of animals, even in the diploblastic coelenterates (Krasińska, 1914) and in phyla like the Mollusca and Annelida where most muscles are smooth, frequently of

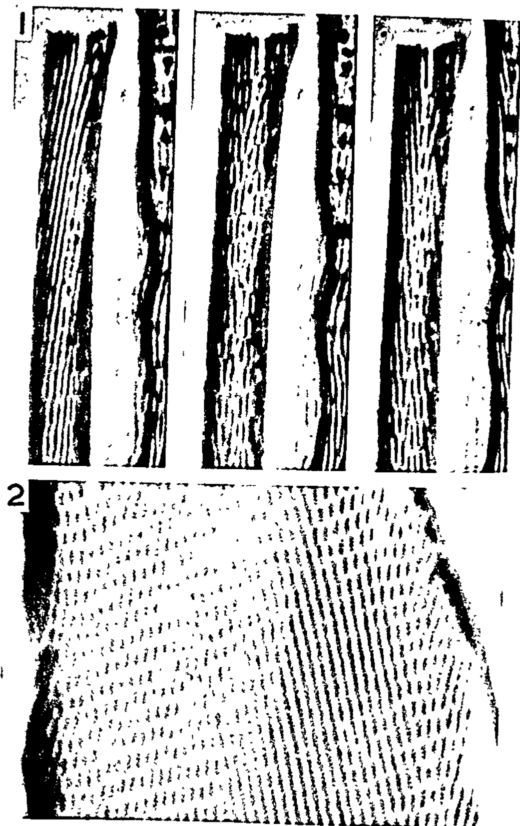


PLATE I

the helical type. In the Chordata and Arthropoda, the somatic and cardiac muscles are striated, almost without exception, and among arthropods the muscles of the alimentary canal also are usually striated.

Other animals with striated muscles are arrow-worms (*Sagitta*, etc.) (Burfield, 1927; H. and L.[1]) which swim with rapid darting movements; certain rotifers (Brakenhoff, 1937) and gastrotrichs (Remane, 1933); Kinorhyncha (Zelinka, 1928); and *Euplocamis* which, unlike other ctenophores, has striated muscles in its tentacles (Biedermann, 1895). Striated muscles are also found in the Bryozoa, examples being the retractors of some cyclostomatous forms (Borg, 1926), which are used for pulling the animal back into its "house," the adductors of avicularia (Délage and Hérouard, 1897), and certain muscles in the free-swimming *Cyphonautes* larvae (Kupelweiser, 1906). Some of the pedicellariae and spines in echinoids also have striated muscles (Hamann, 1887; Kiernick, 1905). It is interesting to find that the adductors of certain pedicellariae consist of two parts, one striated, one smooth (Kiernick). The body wall muscles of *Priapulus* (Apel, 1885) and one of the adductor muscles of the brachiopod *Magellania* (Délage and Hérouard, 1897) are striated. Among the urochordates, muscles which are used for swimming are striated, for instance in salps, *Doliolum*, appendicularians, and tadpole larvae (Kükenthal and Krumbach, 1933).

2. *Helical Smooth Muscles*

Locomotory muscles in annelids and cephalopods have smooth fibers with helically arranged myofibrils. Such fibers also occur in many other molluscs and in other phyla. They are probably much more widespread than is known at present, for they are not always easy to recognize. Examples include some of the intervertebral muscles of ophiuroids (Schwalbe, 1869; Hamann, 1889) and crinoids (Reichensperger, 1912) and certain muscles in the first asexual generation of *Doliolum*

[1] The abbrevation H. and L. throughout this chapter refers to unpublished observations by Hanson and Lowy.

PLATE I. "Helical smooth muscles." Phase contrast photomicrographs of whole isolated fibers. The oblique lines are helically arranged smooth myofibrils.

FIG. 1. Squid (cephalopod mollusc) mantle muscle. Fixed in formaldehyde at approximately maximal body length. The end of a broken fiber photographed at three successive focal levels, the middle one showing "double-oblique-striation." Magnification. × 2,000. (Hanson and Lowy, 1957).

FIG. 2 *Myxicola* (annelid) longitudinal body wall muscle. Glycerol-extracted, unfixed Somewhat contracted. Fiber axis vertical. Magnification: × 4,800.

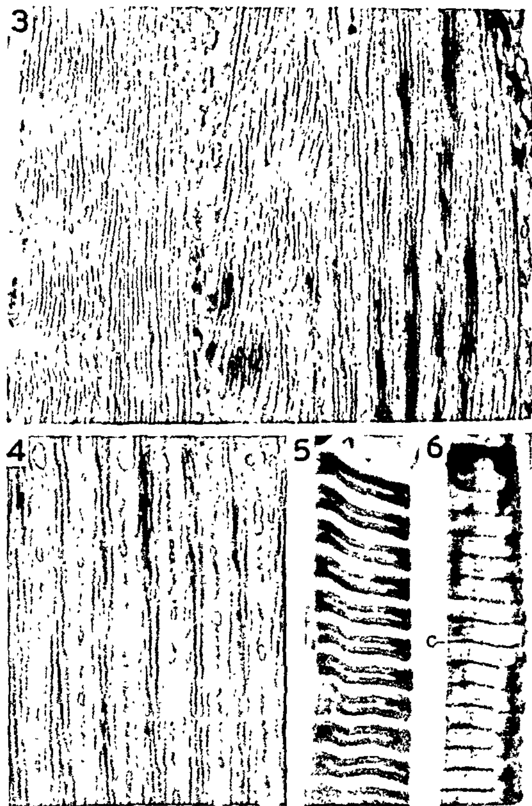


PLATE II

(Grobben, 1881). A double-oblique striation has been reported in the Nematomorpha (Rauther, 1914) but it is unknown if nematodes are similar. Apparently, no arthropods or vertebrates have muscles of this kind.

3. *Paramyosin Smooth Muscles*

The only known examples of this type of muscle occur in molluscs (p 274) where they typically serve a tonic function. They may, however, be much more widespread, for they can only be identified when submicroscopic structure is known.

4. *Classic Smooth Muscles*

Some of the muscles included in this category are fairly well known and appear to be distinct from the smooth muscles of the helical and paramyosin types. The fibers contain small longitudinally oriented filaments, which show no signs of paramyosin structure. This applies to the pharynx and penis retractors of *Helix* (Hanson and Lowy, 1957; Schlote, 1957), to *Phascolosoma* retractors (Fig. 12) (Hanson and Lowy, 1957), and to vertebrate smooth muscles (Caesar *et al.*, 1957, for example). In other cases, however, the term "smooth" is used only in a negative sense, i.e. to indicate that no striations of any kind (transverse or oblique) have been seen. In the absence of further information, nothing positive can be said about such muscle fibers. They are present in all kinds of animals, and no useful purpose will be served by listing examples.

PLATE II.

FIG. 3. "Helical smooth muscle." Earthworm longitudinal body wall muscle. Electron micrograph of longitudinal section through two adjacent fibers. Fiber axis vertical. Glycerol-extracted material. (See Fig. 2 for a light micrograph of a similar fiber.) The fibrils are cut in various planes—some obliquely, giving a false appearance of cross-striation, others longitudinally. Note the sarcoplasmic reticulum, which is shown more clearly in Fig. 4. Magnification: × 20,000.

FIG. 4. Same as Fig. 3 Between the longitudinally cut smooth myofibrils are elements of the sarcoplasmic reticulum. Magnification. × 30,000.

FIGS. 5 and 6. "Striated muscle." Phasic part of the adductor of the scallop *Pecten* Phase contrast photomicrographs of isolated fibers, showing the same bands [illegible] × 2,700.

[illegible] ands and H zones; the [illegible]

FIG. 6. A contracted fiber with contraction bands (C). Fixed in formaldehyde.

TABLE I

A GUIDE TO SOME IMPORTANT FEATURES OF THE FIBERS OF THE BEST-KNOWN MUSCLES

Animal	Group	Muscle	Type of muscle fiber				Presence of two kinds of cross-linked filaments[1]
			Striated	Smooth			
				Helical	Paramyosin	Classic	
Medusae	Cnidaria	Swimming	+				
Phascolosoma	Sipunculoidea	Retractor				+	
Pecten (scallop)	Mollusca (Bivalvia)	Tonic adductor			+		+
Pecten (scallop)	Mollusca (Bivalvia)	Striated adductor	+				+
Oyster	Mollusca (Bivalvia)	Tonic adductor			+		+
Oyster	Mollusca (Bivalvia)	Less tonic adductor			+		+
Squid	Mollusca (Cephalopoda)	Mantle and Funnel retractor		+			+
Helix (snail)	Mollusca (Gastropoda)	Pharynx retractor				+	+
Earthworm	Annelida	Body wall		+			
Blowfly	Arthropoda	Flight	+				+
Sagitta (arrow-worm)	Chaetognatha	Swimming	+				
Amphioxus	Protochordata	Swimming	+				
Rabbit	Vertebrates	Skeletal	+				+
Rabbit	Vertebrates	Cardiac	+				+[2]
Rabbit	Vertebrates	Visceral				+	

[1] This indicates that the muscle may contract by a sliding filament mechanism.
[2] Unpublished results of H. E. Huxley and R. Niedergerke.

D. Survey of the Better Known Phyla

1. *Cnidaria*

Many of the animals in this phylum are sessile polyps, like *Hydra*, anemones, and corals, while others are motile medusoid forms, like jellyfish. Medusae swim with fairly rapid rhythmic movements (Romanes, 1885) and their swimming muscles are striated (Krasińska, 1914; Horridge, 1954). Polyps, on the other hand, have "classic" smooth muscles (see Hess *et al.*, 1957, for a recent study of *Hydra*) and they change shape rather slowly; retractor and sphincter muscles are sometimes differentiated, but these also are smooth.

2. *Nematoda*

The very large muscle cells in the body walls of nematodes (roundworms) have often been studied and indeed are described in most textbooks, but it is surprising to find how little of significance is known about them (Apathy, 1893; Roskin, 1925 b; Mueller, 1929). As in many other invertebrates, the nucleus is situated in a "cell body" lying to one side of the contractile fibrous part of the muscle cell. The contractile elements, which are smooth, are lamellar in shape and composed of small "fibrils," and they appear folded in various ways when viewed in cross section. A rather complicated system of intracellular "fibers," situated between the lamellae, is seen when sectioned material is viewed in the light microscope, and has in the past been interpreted in several different ways. It would be interesting to know if these "fibers" are in fact elements of a sarcoplasmic reticulum of exceptionally large size, commensurate with the very large scale of the whole cell. Nematodes, even some parasitic ones, often move very actively.

3. *Sipunculoidea*

This small phylum includes *Sipunculus* and *Phascolosoma*, animals in which the front part of the body can be drawn back by four smooth retractor muscles, which can undergo a wide range of length changes, and have been the subject of several recent physiological and other investigations. The relations between length and birefringence have been studied (Fischer, 1947) and are discussed later (p. 285). The wide-angle X-ray diffraction pattern is of the same type as in striated muscles and some other smooth muscles, and it changes little with muscle length (Astbury, 1947). Electron micrographs show that the

fibers, which are about 5 μ in diameter, contain longitudinal protein filaments, apparently *all* of the same kind, not arranged in any regular manner, and not grouped into fibrils (Hanson and Lowy, 1957) (Fig. 12). It has been reported (Bear, 1945; Schmitt *et al.*, 1947) that the low-angle X-ray diffraction pattern of *Phascolosoma* muscles has reflections attributable to paramyosin, although the strongest components are those due to actin. Only the latter were found in *Dendrostomum* (Worthington, 1956). Elements showing paramyosin structure have not yet been seen in electron micrographs.

4. *Mollusca*

All the main types of muscle fibers are represented in this phylum. The most interesting muscles are the adductors of lamellibranch (bivalve) molluscs, which serve to close the shell and maintain tension against a springy hinge ligament. Generally, an adductor consists of two parts, one of which is white and opaque and very specialized to serve a tonic function, i.e. for the prolonged maintenance of a high level of tension. The other part is more translucent and often colored, and is less specialized for tonic function. The more tonic muscle is always a smooth muscle of the paramyosin type (Figs. 13–17) (Bear, 1944; Hall *et al.*, 1945; Schmitt *et al.*, 1947; Elliott *et al.*, 1957). The other muscle varies greatly from one species to another. In a few cases, it is striated (Figs. 5 and 6), for instance in the scallop, *Pecten*, where it is used for swimming. The low-angle X-ray diffraction pattern of this striated muscle is like that of a vertebrate skeletal muscle, no paramyosin reflections being present (Bear, 1945; Schmitt *et al.*, 1947). In electron micrographs, also, it looks like vertebrate and arthropod muscles, being composed of alternating and inter-digitating arrays of two types of protein filaments (H. and L.).

In most lamellibranchs the translucent (less tonic) part of the adductor is a smooth muscle. In some (eg. *Pinna*, *Mytilus*, *Ostrea*, *Gryphaea*, *Venus*, *Anodonta*) there is already information from X-ray diffraction patterns (Schmitt *et al.*, 1947) and/or electron micrographs (H. and L.) that it is a "paramyosin smooth muscle".

In some species, for instance in *Cardium* and *Cyprina*, although the fibers from the two parts of the adductor look alike within the range of lengths they can assume in the body, the less tonic fibers develop "contraction bands" if detached from the shell and allowed to shorten

excessively (Hanson and Lo[illegible] bands are often helically arranged, giving a [illegible] double-oblique striation" to the fiber.

Fibers with helically-wound smooth myofibrils are characteristic of nearly all the muscles of cephalopods (Fig. 1), including not only the powerful mantle muscles, which are used for respiration and swimming, but also the muscle coats of the alimentary canal (H. and L.). The cardiac muscles, however, are striated, as they also are in some lamellibranchs and gastropods (Marceau, 1905b; Krijgsman and Divaris, 1955; H. and L.). Other molluscan cardiac muscles appear to be of the helical smooth type (Marceau, 1905b), and some are of the classic smooth type (Motley, 1933; Esser, 1934). It is curious to find that the radial muscles in the long arms of female decapods are striated (H. and L.), while in the shorter arms and in the male they are of the smooth (helical) type. In the eye of *Sepia*, there are some small striated muscles associated with the retina (Alexandrowicz, 1927).

The myofibrils in the mantle and in the funnel retractor of the squid are constructed, along the whole of their length, of two types of cross-linked protein filaments, and resemble the A bands of striated myofibrils (Hanson and Lowy, 1957) (Figs. 7 and 8).

So far we have mentioned three types of molluscan muscle fibers, striated, "helical," and "paramyosin." A smooth muscle of different construction can also be distinguished. In the edible snail, *Helix pomatia*, the pharynx retractor muscle, which has been the subject of several physiological studies, consists of smooth fibers containing longitudinal filaments of two kinds; these filaments are cross-linked together in the same way as in striated muscles; they are not grouped into myofibrils, and they are irregularly packed with respect to each other (Hanson and Lowy, 1957). The penis retractor of the same species is similarly constructed (Schlote, 1957).

To conclude this account of molluscan muscles, a few other facts are worth noticing here. The paramyosin type of smooth muscle fiber is not confined to the adductors of lamellibranchs, but is also found in the byssal and pedal retractors of *Mytilus* (G. F. Elliott, and H. and L., unpublished) and probably in the foot retractors of the snails *Viviparus* (Hall *et al.*, 1945) and *Helix* (Hanson and Lowy, 1957), from both of which ribbon-shaped paramyosin elements have been isolated. The rhythmically contracting respiratory septum in *Cuspidaria* and *Poromya* has striated fibers (Yonge, 1928). In the pteropods (planktonic swim-

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PLATE III

ming gastropods), the muscles of the "wings" are sometimes of the smooth helical type (Roskin, 1925a), sometimes striated (H. and L.). In a number of gastropods, the radular muscles and tentacle retractors are striated (Merton, 1911).

5. *Annelida*

The somatic muscles of annelids consist of smooth fibers in which the fibrils are helically arranged (Figs. 2–4). "Double oblique striation" was first observed by Mettenheimer (1860) in *Arenicola*. [In the same year it was observed in *Octopus* by Margo.] As in the case of molluscs, the interpretation of this "striation" has been much debated (Prenant, 1929), but recent studies on the earthworm, *Lumbricus*, using phase contrast and electron microscopy, have shown clearly that the lines of the diamond lattice pattern are smooth myofibrils (Hanson, 1957). The body wall muscles of many annelids are capable of fairly rapid movements, in many cases being stimulated by giant nerve fibers; the latter are particularly well-developed in serpulids (Nicol, 1951).

These helical muscles of oligochaetes and polychaetes differ from those of molluscs in that they are often very flat, wide in one dimension (about 30 μ in *Myxicola*, for example, H. and L.), but very thin in the other (less than 5 μ). In leeches, on the other hand, as in cephalopods, the fiber is more nearly circular in cross-section, and there is a thick core of nonfibrillar cytoplasm (Schwalbe, 1869).

There are few authentic cases of striated muscles in annelids. Among them are the pharyngeal muscles of syllids, some of which have sarcomeres 33 μ long (Haswell, 1889; Schmidt, 1936), certain head muscles in a syllid (Prenant, 1912), and an interesting and little known system of small muscle fibers in *Nephthys* (Emery, 1887).

In comparative studies on the tropomyosins of invertebrate animals, Kominz *et al.* (1957a, 1958) have discovered that annelid muscles contain the same type of tropomyosin as that which is present in molluscan paramyosin muscles, where it is believed to be responsible for paramyosin structure (see p. 297). One might expect that annelid muscles

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PLATE III. "Helical smooth muscle." Squid mantle muscle. Electron micrographs of sections through glycerol-extracted fibrils (Hanson and Lowy, 1957).

FIG. 7. Transverse section. The fibrils are lamellar in shape. A double array of filaments—thick and thin—is seen. Magnification: × 160,000.

FIG. 8 Longitudinal section. Cross-links between thick and thin filaments are seen. Magnification: × 160,000.

would also contain paramyosin elements, and recently an axial periodicity like that of paramyosin has been noticed in the filaments of *Lumbricus* muscles (Hanson and Lowy, 1957).

6. *Arthropoda*

As in vertebrates, the somatic muscles of arthropods work on a hard skeleton and are striated. The idea that striation is in some way correlated with rapidity of movement is, however, not immediately reconcilable with the fact that the muscles of the alimentary canal, in arthropods, are generally striated (Wigglesworth, 1950).

There are exceptions to the general rule that all arthropod muscles are striated. As Darwin (1851) observed, the peduncle muscles of barnacles are smooth, and this applies also to the mantle and adductor muscles in some species (Koehler, 1889; Gruvel, 1896).

In the Onychophora (*Peripatus*, etc.) and Tardigrada, two groups which are allied to arthropods, the somatic muscles are smooth (H. and L. for *Peripatus*; Plate (1888) for tardigrades) and they work on a deformable connective tissue.

7. *Chordata*

This phylum includes the vertebrates and some other classes which lack vertebral columns.

a. Protochordates. Amphioxus, like the vertebrates, has striated somatic muscles (Schneider, 1902; H. and L.). Hemichordates (*Balanoglossus*, etc.) appear to have smooth muscles. Among the urochordates (reviewed in Kukenthal and Krumbach, 1933), the somatic muscles of the sessile forms (ascidians) are smooth ("classic"), but the swimming muscles of the motile forms (salps, *Doliolum*, appendicularians, tadpole larvae) are striated. The ascidian heart has striated muscles (Krijgsman, 1956), and Bozler (1928) concluded that individual muscle fibers contain both smooth and striated contractile elements; this extremely interesting observation awaits confirmation.

b. Vertebrates. In general, the somatic and cardiac muscles are striated and the visceral muscles are of the classic smooth type. But the upper part of the mammalian esophagus has striated muscles, and in the intestine of the tench there is a coat of striated muscles as well as a coat of smooth muscles (Retterer and Lelièvre, 1909). So far as is

known, neither "paramyosin smooth muscles" nor "helical smooth muscles" are found in vertebrates.

III. STRUCTURE

A. Structures other than the Contractile Apparatus

1. *Size and Nuclei*

Striated muscle fibers are usually multinucleate and frequently much larger than smooth ones. As far as is known, smooth fibers are always uninucleate and rarely more than 10–15 μ in diameter. Although in many annelids one dimension of the lamellar-shaped smooth fiber is large, the other dimension is only about 5 μ (H. and L.). Nevertheless, some smooth muscle fibers are quite long, up to 2 cm. in the anterior byssal retractor of *Mytilus*, for example (Fletcher, 1937), and about the same in the pharynx retractor of *Helix* (Bozler, 1930). Exceptionally large smooth muscle cells are found in nematodes, where the whole animal consists of very few cells. In *Ascaris*, the fibrous part of the cell, in cross section, measures as much as 1 mm. × 0.2 mm., and the "cell body" is as large (H. and L.). At the other extreme, there are many rotifers which measure much less than 1 mm. in length (Wesenberg-Lund, 1939) and yet have numerous discrete muscles (striated and smooth).

Some of the thickest striated muscle fibers, about 2 mm. in diameter, are to be found in the indirect flight muscles of Diptera (Tiegs, 1955), and one wonders how stimuli are conducted to the center of the fiber. Possibly this process is aided by invaginations of the excitable membrane around the tracheoles which penetrate into the fiber; in the leg muscles of a wasp, it has been shown that the plasma membrane is thus invaginated (Edwards *et al.*, 1958) but it is not yet known if this occurs in flight muscles. Intracellular nerve endings have been described in dipteran flight muscles (Tiegs, 1955), but perhaps what really happens is that nerves accompany the tracheoles, so that the endings are not in fact intracellular.

2. *Sarcolemma*

The question of the presence or absence of a sarcolemma in insect muscles has been much debated. However, the existence of a fine continuous membrane, presumably the excitable membrane, is now known from electron micrographs (Chapman, 1954; Hodge, 1955; Edwards

et al., 1956, 1958). The nature of the connective tissue component is still not understood. Collagen fibers are absent and, in fact, arthropods apparei
cuticle-
tissue which holds the muscle fibers together (Edwards *et al.*, 1958), or that amebocytes lay down a kind of connective tissue (Wigglesworth, 1956).

3. *Sarcoplasmic Reticulum*

A sarcoplasmic reticulum seems to be present in all types of muscle fibers. Its detailed anatomy has not yet been described in any invertebrate muscle. A high degree of organization is clearly present in the reticulum of earthworm fibers (Figs. 3 and 4) (Hanson, 1957; Hanson and Lowy, 1957; Edwards, 1957) and squid fibers (Hanson and Lowy, 1957), both of which are of the helical type. The presence of a reticulum in the "classic" smooth fibers of the penis retractor of *Helix* has also been noted (Schlote, 1957). In view of the suggestion that the reticulum is concerned with the conduction of excitation (see Volume I, Chapter VI), it is very interesting to find that it is better developed in the leg muscles of insects than in their specialized flight muscles (Edwards *et al.*, 1956).

4. *Sarcosomes*

In the flight muscles of insects, it is known that enzymes concerned with oxidative metabolism are situated in the sarcosomes (mitochondria) and that enzymes for anaerobic glycolysis are present in solution in the sarcoplasm (Watanabe and Williams, 1951; Sacktor, 1955; discussion in Pringle, 1957). This is true of other muscles (Volume II, Chapter III), and it is significant that those vertebrate skeletal muscles which accumulate an oxygen debt while they are working have relatively small sarcosomes, while cardiac muscles have many large ones (Cleland and Slater, 1953). The sarcosomes are very numerous and reach a large size (Fig. 19) in the flight muscles of those insects which are good fliers, such as flies, midges, bees, wasps, and locusts (Tiegs, 1955); in *Phormia*, they may account for as much as 40% of the weight of the muscle (Levenbook and Williams, 1956). As Keilin (1925) discovered, such muscles have more cytochrome than any other living system. It is interesting to find that in flies, during the first few days after emergence from the pupa, while the frequency of wing beat is

increasing, the size and the cytochrome content of the sarcosomes, and their rate of oxidative phosphorylation, show a parallel increase (Keilin, 1925; Lewis and Slater, 1954; Levenbook and Williams, 1956). In the adult insect (flies and locusts), oxygen is used at a very high rate during flight (Weis-Fogh, 1952; Chadwick, 1953), and even after long periods only a negligible oxygen debt is accumulated (Chapman and Gilmour, 1940; Krogh and Weis-Fogh, 1951). Evidently, as Buchthal *et al.* (1957) point out, complete recovery is included within the period of mechanical response in locust flight muscle, which never contracts tetanically in the living animal, but works by repetitive twitches, wing stroke frequency being equal to twitch frequency (Weis-Fogh, 1956b).

Oxygen is brought to the sarcosomes by tracheoles (Hodge, 1955; Edwards *et al.*, 1956, 1958). The presence of tracheal branches inside muscle fibers was observed in 1859 by Leydig. The leg muscles of insects have a poorer tracheal supply and smaller sarcosomes, and the same is true of the flight muscles of insects like cockroaches (Edwards *et al.*, 1956).

These well-known facts about insect muscles lead one to inquire about the sarcosomes in other invertebrates. They are very large in the mantle fibers of cephalopods, where they are situated in a central core surrounded by the helically wound fibrils (Hanson and Lowy, 1957). Cephalopods are very active animals and use the mantle for swimming and respiration. In contrast, the tonic fibers of lamellibranch adductors seem to have only a few very small sarcosomes (H. and L.); as we shall see later, these muscles maintain tension for long periods with very little expenditure of energy.

It is unfortunate that more is not known about the quantity and size of sarcosomes in other invertebrate muscles, for these may be major factors controlling the mode of action of a muscle, perhaps just as important as the details of construction of the contractile apparatus.

B. Contractile Apparatus

1. *Filaments and Fibrils*

In all muscles, the contractile apparatus is made up of protein filaments which are too small to be resolved in the light microscope, but can be seen in electron micrographs. Such filaments are present in contractile cells in which the light microscope detects no fibrillar material, for instance in embryonic myoblasts (Hibbs, 1956). In adult

et al., 1956, 1958). The nature of the connective tissue component is still not understood. Collagen fibers are absent and, in fact, arthropods apparently do not possess collagen. It has been suggested that the cuticle-like basement membranes of tracheoblasts provide a connective tissue which holds the muscle fibers together (Edwards *et al.*, 1958), or that amebocytes lay down a kind of connective tissue (Wigglesworth, 1956).

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fibers of many kinds, parts of the sarcoplasm appear in the light microscope to be nonfibrillar, and Hoyle (1957) has recently revived the old suggestion (Bottazzi, 1897) that this undifferentiated sarcoplasm may be contractile, serving a tonic function, while the fibrillar parts of the cell are used for phasic contractions only. In the relatively few muscles which have been examined by electron microscopy, no filaments have been found in the undifferentiated sarcoplasm. Moreover, in many such muscles, it is clear, even in the light microscope, that this part of the sarcoplasm is full of sarcosomes and is therefore much more likely to serve a metabolic function than to be contractile.

Many smooth muscle fibers show a more or less homogeneous appearance in the light microscope, provided that they have not been damaged by fixation or dehydration. This is due to the fact that their filaments are not grouped into well-differentiated fibrils, but are present over the whole cross-sectional area of the fiber. Examples of such muscles are the retractors of *Phascolosoma* (Fig. 12), the pharynx and penis retractors of *Helix*, and also vertebrate smooth muscles (Hanson and Lowy, 1957; Schlote, 1957). "Paramysion muscles" appear fibrillar in the light microscope (Fig. 10) because of the large size of their filaments, which may be as much as 1500 A. in diameter.

2. *The Contractile Unit and its Construction*

a. Introduction. The question of what is the smallest contractile unit in a muscle fiber, whether a single filament, or a particular grouping of

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PLATE IV.

FIG. 9 Phase contrast photomicrographs of fibers from the less tonic (translucent) part of the adductor muscle of *Cyprina*. Whole fibers, fixed in formaldehyde. Magnification: × 2,700. (a). Fixed at maximal body length. (b). Fixed after excessive contraction to less than minimal body length. Note the "double-oblique" pattern of "contraction bands" superimposed on longitudinal lines like those seen in (a).

FIG. 10 "Paramyosin smooth muscle." The opaque (tonic) part of an oyster adductor muscle. Phase contrast photomicrograph of an isolated fiber fixed in formaldehyde at maximal body length. The longitudinal lines are not fibrils, but are due to the large size of the filaments (see Plate V). Magnification: × 2,400.

FIG. 11. Electron micrograph of a transverse section through parts of two adjacent fibers, in the translucent (less tonic) part of an oyster (*Ostrea*) adductor muscle, showing the regularly arranged filaments in the lamellar-shaped fibrils. Glycerol-extracted material. Magnification· × 20,000. (Hanson and Lowy, 1957).

FIG. 12. "Classical smooth muscle." Retractor of the sipunculid *Phascolosoma*. Electron micrograph of a longitudinal section. The filaments are parallel to the fiber axis and are not grouped into fibrils. Magnification: × 20,000.

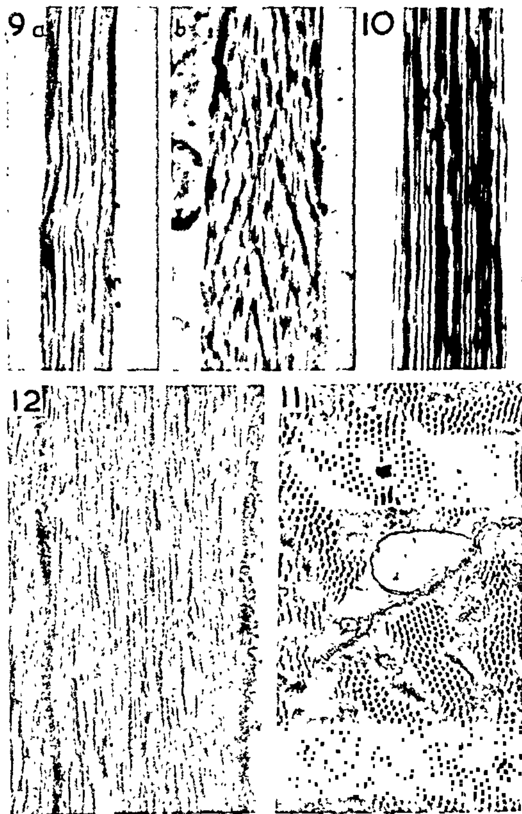


PLATE IV

the penis retractor of the dog (Bear, 1945). As yet, no detailed study appears to have been made on the effects of muscle length on these actin patterns in smooth muscles, but in the case of the *Venus* adductor the pattern seems to remain unchanged when the length of the muscle is altered (Selby and Bear, 1956). Hence there is no evidence from X-ray diffraction data that individual *filaments* in smooth muscles shorten on contraction. But a much more thorough study is needed (see Appendix). It must also be remembered that paramyosin structure is very probably due to tropomyosin (see p. 297), and as yet there is no evidence that tropomyosin participates in contraction in any muscle.

Brief mention should be made here of a remarkably detailed wide-angle X-ray diffraction pattern, known as the "Lotmar-Picken pattern," which has occasionally been obtained from various muscles, and particularly investigated in invertebrates (Lotmar and Picken, 1942; Bear and Cannon, 1951; Huxley and Kendrew, 1952; Worthington, 1956). This pattern is now known to be due to the amino acid taurine (Cohen, 1958) which is readily washed out of the muscle.

c. Birefringence. Measurements of the relative contributions of form and intrinsic components to total birefringence are difficult to make and to interpret. However, studies on the smooth ("classic") retractors of *Thyone* and *Phascolosoma* have shown that changes in instrinsic birefringence (presumably structural alterations inside filaments) contribute rather little to the marked changes which take place in total birefringence when the length of the muscle is altered (Fischer, 1947). These results are not inconsistent with the idea that the length of individual filaments does not change appreciably with changes in muscle length.

d. Striated muscle. The striated fibrils of two different invertebrate muscles have been examined by electron microscopy, and it has been found that they have essentially the same structure as fibrils in vertebrate skeletal muscles; this applies to the indirect flight muscles of *Calliphora* (Huxley and Hanson, 1957) and to the phasic adductor of *Pecten* (H. and L.). In all cases, vertebrate and invertebrate, the band pattern which is visible in the light microscope undergoes the same changes on contraction, and this is also true of *Cuspidaria* septal muscles (H. and L.). It can be concluded that the mechanism of contraction, in principle, is probably the same in all striated muscles. Moreover, structural considerations suggest that certain smooth muscles may also contract by a sliding filament mechanism.

filaments, has recently come into prominence with the discovery that in striated muscles the filaments are very much shorter than the length of the fibril, and appear to slide past each other during contraction, without necessarily undergoing any overall changes in length (see Volume I, Chapter VII). The observations which led to this conclusion were greatly facilitated by the fact that the positions of the filaments are reflected in the striations. Since these are visible in the light microscope, observations made *during* contraction can give information about the behavior of the submicroscopic filaments. The results of such observations helped to explain the discovery, made by low-angle X-ray diffraction methods (H. E. Huxley, 1951, 1953), that the 415 Å axial periodicity in striated muscle does not change with the length of the muscle. They also shed new light on the fact that the wide-angle X-ray diffraction pattern changes very little (Astbury, 1947). (Wide-angle patterns give information about intramolecular architecture, low-angle patterns about structure on a larger scale, up to about 0.1 μ.)

In the case of smooth muscles, one cannot straightforwardly deduce anything about the behavior of the filaments from studies on contracting fibers or fibrils viewed in the light microscope, and for various technical reasons it is virtually impossible to measure filament lengths by electron microscopy. However, X-ray diffraction patterns can be obtained.

b. X-ray Diffraction. The wide-angle pattern of the byssal retractor of *Mytilus* (a "paramyosin muscle") or of *Phascolosoma* retractors ("classic" smooth muscles) is essentially the same as that of striated muscle and shows no significant changes on contraction (Astbury, 1947).

Low-angle patterns from unstriated muscles are of two kinds. Paramyosin muscles give a pattern which is dominated by reflections from the paramyosin structures (Bear, 1944, 1945) (Fig. 16). When such muscles were extended or contracted and then dried and examined, no changes were observed in axial periodicities (Bear, 1944; Bear and Selby, 1956). Similarly, the spacings in paramyosin filaments examined in the electron microscope showed no changes (Hall *et al.*, 1945). Other smooth muscles give a low-angle pattern which is like that of striated muscle and is attributed to actin. This applies to the translucent (less tonic) part of the adductor in some lamellibranchs (Bear, 1945; Selby and Bear, 1956) and to other invertebrate smooth muscles,—*Phascolosoma* and *Thyone* retractors (Bear, 1945; Schmitt *et al.*, 1947), and *Dendrostomum* retractors (Worthington, 1956),—and also to

e. Smooth muscles with two types of filaments. As we have seen, the pharynx retractor of *Helix*, and the funnel retractor and mantle muscles of the squid have two types of filaments, cross-linked by a series of bridges which look very like those present in striated muscles (Hanson and Lowy, 1957) (Fig. 8). In the squid, a transverse section through any part of a fibril shows a hexagonal array of thick filaments, about 120 Å. in diameter, and between them a number of thinner filaments, about 50 Å. in diameter (Fig. 7). These two kinds of filaments closely resemble those of striated muscles in diameter and arrangement. It is not known, however, if the larger filaments contain myosin and the smaller one actin, as they do in vertebrate muscles. The penis retractor of *Helix* also contains thick and thin filaments (Schlote, 1957) arranged as in the pharynx retractor. Dr. Richard L. Wood (personal communication) has also observed the presence of two types of filaments in the muscles of *Hydra* (a coelenterate) and *Dugesia* (a flatworm). More examples are given in the Appendix.

f. Paramyosin smooth muscles[1]. A paramyosin muscle fiber (for example from the specialized tonic part of a lamellibranch adductor muscle) contains numerous irregularly arranged longitudinal filaments of a wide range of diameters (150–1500 Å) and of complex structure (Figs. 13-15). Studies on these filaments have led to the conclusion that each consists of a number of ribbon-shaped paramyosin elements, stacked longitudinally and surface to surface. Such elements can easily be isolated from mechanically disintegrated muscles (Fig. 17) and were described several years ago (Jakus *et al.*, 1944; Hall *et al.*, 1945). The mechanism of contraction in these interesting muscles is open to speculation; perhaps the filaments are in fact fibrils in which the paramyosin ribbons are cross-linked and slide past each other during contraction. This raises

[1] See Appendix

PLATE V "Paramyosin smooth muscle." Electron micrographs of sections through fibers from the opaque (tonic) part of the oyster adductor muscle (Taken by G. F. Elliott).

FIG. 13 An accurately oriented transverse section showing filaments apparently composed of lamellar-shaped subunits. Magnification: × 92,000.

FIG. 14 Longitudinal section showing the complex and variable structure of the filaments; these are thought to consist of ribbon-shaped paramyosin elements, one of which is shown in Fig. 17. Magnification: × 78,000.

FIG. 15 Same as Fig. 14, except that some protein has been extracted from the fiber, the structure of the filaments is now seen more clearly. Magnification: × 55,000.

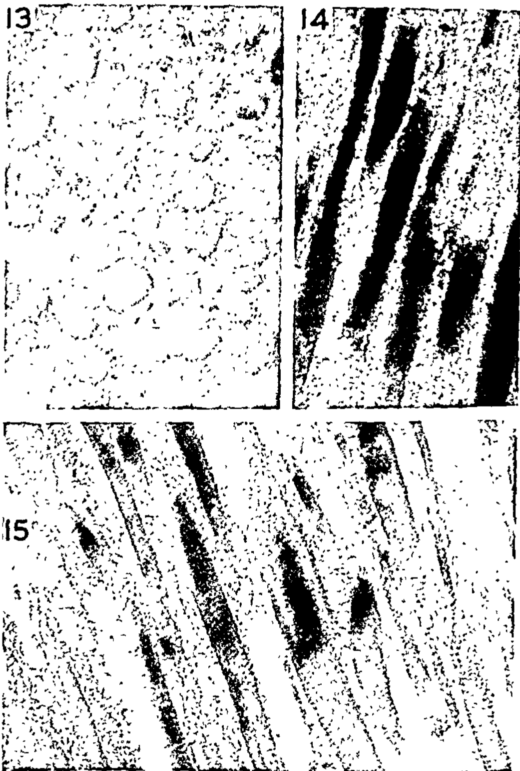


PLATE V

with "double-oblique striation" is controversial. Some authors, in particular Plenk (1924, 1925a, b, 1933) and Schmidt (1938) held the view that such muscles are in reality cross-striated; others (Engelmann, 1881; Fol, 1888; Ballowitz, 1892; Marceau, 1905a, b, 1909) maintained that they are muscles with helically arranged fibrils. No doubt many difficulties arose because the material was fixed and stained, and observed in unsuitable ways. However, studies on whole, unfixed, glycerol-extracted fibers in a phase contrast microscope give unambiguous results (Fig. 1), and low power electron microscopy overcomes the difficulty that in some of these muscles, particularly those of annelids, the light microscope is almost inadequate to resolve the fibrils. Such methods show clearly that the fibrils are smooth and are helically arranged in those muscles which exhibit the diamond lattice pattern at physiological lengths (Hanson, 1957; Hanson and Lowy, 1957).

IV. BIOCHEMISTRY

A. Structural Proteins

1. *Introduction*

The contractile elements of all kinds of muscles seem to consist mainly of actomyosin and tropomyosin. These proteins have been identified in a wide range of muscles and in many kinds of animals. The criteria for recognizing them, however, are somewhat unsatisfactory, nor is it by any means certain that they exist as these entities in the living muscle.

The greater part of our knowledge of these proteins comes from studies on the skeletal muscles of rabbits, and here it is usually recognized that actomyosin is a complex of two proteins, actin and myosin, which are situated in different filaments in the myofibrils, and interact with each other and with ATP to bring about contraction (see Volume I, Chapter VII). Turning to other muscles, evidence for the existence of actin and myosin is not as substantial as one would wish. No detailed accounts have yet been published of the preparation or of the properties of actin or of myosin from any smooth muscle. [But there are reports that actin has been made from the oyster adductor (Bailey, 1956) and myosin from the *Pecten* adductor (Ruegg, 1957).] The evidence that actin and myosin are present in muscles other than vertebrate skeletal ones rests mainly on the fact that it is possible to extract a protein which significantly resembles rabbit actomyosin in the following properties:

the problems of the location of actomyosin and of the function of tropomyosin, which is quantitatively the more important protein in these fibers (Bailey, 1956, 1957; Ruegg, 1957; H. and L.) and appears to be responsible for the fine structure of the paramyosin elements (Hanson *et al.*, 1957; Elliott *et al.*, 1957) (see p. 297).

C. Helical Organization in Muscle Fibers

Two distinct types of helical organization in muscle fibers have been described. In one type which, as far as we know, is found only in certain smooth muscles of invertebrate animals, the myofibrils and their filaments follow a helical course in the muscle fiber. Theoretically, this arrangement could enhance the extent to which the fiber shortens for a given shortening of its fibrils (Marceau, 1909) and this may be of importance in some cases. However, measurements of the angle subtended between the helix and the fiber axis in squid muscles at maximal body length (Hanson and Lowy, 1957) have shown that here it is small (5–10°)—too small to have much effect. When such muscles shorten, the angle increases, and in glycerol-extracted fibers treated with ATP, it may become as large as 60°.

Secondly, in some other muscles, it appears that the contractile elements lie parallel to the fiber axis, but certain features of their axial differentiation lie in register in such a way as to impose a helical organization on the fiber as a whole. Thus in a striated muscle of this type, the *bands* of the fiber will follow a helical course. Convincing descriptions have been given (Tiegs, 1955) of fibers with helically arranged bands, in both vertebrates and arthropods. Tiegs points out that Leeuwenhoek probably noticed the same phenomenon. The question arises whether or not any striated muscle fiber *in vivo* actually has a helical organization of this kind. The fact that the configuration is helical and not random favors the view that it is not an artifact.

In certain smooth muscles, for instance in the translucent (less tonic) parts of some lamellibranch adductors, helically-arranged dense lines develop on excessive shortening, and appear to be superimposed on longitudinal contractile elements (Hanson and Lowy, 1957) (Fig. 9). The axial separation of these lines is approximately 5 μ. Little is known about the structures which are responsible for these "contraction bands." Perhaps they mark the extremities of filaments, as do the contraction bands of striated muscles.

We have not yet mentioned that the earlier literature on muscles

TABLE II
ACTOMYOSINS PREPARED FROM DIFFERENT INVERTEBRATE MUSCLES

Phylum	Genus	Muscle	Type	References
Cnidaria	*Anthopleura*	Whole body	Smooth (Classic)	1, 2, 3
Sipunculoidea	*Urechis*	Body wall	Smooth (Classic)	4
Mollusca	*Pecten*	Tonic adductor	Smooth (Paramyosin)	5, 6, 7
		Striated adductor	Striated	5, 6, 7, 8
	Anodonta	Foot	Smooth	10
	Squid	Mantle	Smooth (Helical)	11
	Murex	Foot	Smooth (Paramyosin?)	12
Annelida	*Lumbricus*	Body wall	Smooth (Helical)	13
Arthropoda	Lobster	Abdominal	Striated	14
	Crayfish	Abdominal	Striated	15
	Limulus	Longitudinal	Striated	16,17
	Locust	Leg and Flight	Striated	18
	Bee	Flight	Striated	19
Echinodermata	*Asterias*	Tube feet	Smooth (Classic)	20

References: 1. Maruyama (1955) 2. Maruyama (1958a, b) 3. Maruyama (1958a, b) 4. Maruyama (1954a) 5. Tonomura *et al.* (1955, 1956) 6. Tonomura *et al.* (1955, 1956) 7. Ruegg (1957) 8. Matsumiya *et al.* (1957) 9. Yagi (1957) 10. Dorr and Portzehl (1954) 11. Villafranca (1955) 12. Dubuisson and Pezeu (1947) 13. Godeaux (1954) 14 Dubuisson-Brouha (1953) 15. Maruyama (1958c) 16. Sarkar (1951) 17. Amberson *et al.* (1949) 18. Gilmour and Calaby (1953) 19. Maruyama (1957) 20. Maruyama and Matsumiya (1957).

Although several studies have been made on the properties of these actomyosins, including the effects of ATP on the viscosity and turbidity of solutions, no differences have yet been established which might help us to understand the functional or the structural differences between muscles.

Tonomura and his colleagues (Tonomura, 1956) have suggested that there is a significant difference between smooth and striated muscles in the number of ATP-binding sites in the actomyosin molecule, and hence, by inference, in the number of sites which may be active in contraction. However, results obtained by Ruegg (1957) indicate that the preparations of actomyosin made by Tonomura *et al.* (1955, 1956) from the smooth adductor of *Pecten* almost certainly contained enough tropomyosin to account for the difference observed between this muscle and the striated adductor of *Pecten*.

3. *ATPase Activity of Actomyosin*

The ability of myosin to catalyze the removal of the terminal phosphate group from ATP is of outstanding importance, for it seems pos-

(*a.*) The viscosity and the turbidity of solutions of this protein decrease on addition of ATP, and then tend to recover their original values as the ATP is dephosphorylated. This behavior is believed to be due to the dissociation of actomyosin into its two components in the presence of ATP.

(*b.*) The precipitated protein exhibits syneresis when treated with ATP, and threads of it shorten.

(*c.*) The ATPase activity of this protein is influenced in much the same manner as is the activity of rabbit actomyosin by several factors, such as divalent cations and total salt concentration.

The myosin of rabbit skeletal muscles can be split into two distinct parts, known as the "heavy" and "light" meromyosins (Volume II, Chapter I). Needham and Cawkwell (1958) have now reported the preparation of heavy acto-meromyosin from a mammalian smooth muscle. But meromyosins have not yet been prepared from any invertebrate muscles.

Tropomyosin can be identified by its amino acid composition, which, however, varies slightly from one muscle to another, as do some other properties. Two main types of tropomyosin are recognized (Bailey, 1956, 1957; Kominz *et al.*, 1957a, 1958), and much of our knowledge about them comes from recent work on invertebrate muscles.

Although these different proteins are readily distinguished in extracts made from the contractile elements of muscles, their existence in life is only inferred from indirect evidence. The most satisfactory kind of evidence is that which actually locates different proteins in different structures. For striated muscles, but for no others, information of this kind exists, as far as actin and myosin are concerned. Tropomyosin presents a major unsolved problem, for nothing is known about its function. Studies on invertebrate muscles will probably help to solve this question, for in some of them it seems that there is more tropomyosin than actomyosin, and, moreover, such muscles have special physiological properties.

2. *Actomyosin*

Proteins resembling the actomyosin of rabbit skeletal muscles have been obtained from all the main types of muscles and from representatives of several phyla (Table II).

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3. *ATPase Activity of Actomyosin*

The ability of myosin to catalyze the removal of the terminal phosphate group from ATP is of outstanding importance, for it seems pos-

sible that this reaction releases the energy which is used in contraction. The question arises whether or not the inherent enzymatic activities of the myosins of different muscles are in any way correlated with particular physiological properties.

The ATPase activities of the myosins of invertebrate animals have not yet been studied. At first sight (Table III), it appears that the

TABLE III

VALUES REPORTED FOR THE ATPASE ACTIVITIES OF ACTOMYOSINS PREPARED FROM DIFFERENT MUSCLES

Phylum	Genus	Muscle	Type	Qp *a*	References
Sipunculoidea	*Urechis*	Body wall	Smooth (Classic)	1300	1
Mollusca	*Pecten*	Tonic adductor	Smooth (Paramyosin)	260	2
	Pecten	Tonic adductor	Smooth (Paramyosin)	2700	3
	Pecten	Striated adductor	Striated	780	2
	Pecten	Striated adductor	Striated	2700	3
	Mya	Adductor	Smooth (Mixed types)	9	4
	Saxostrea	Tonic adductor	Smooth (Paramyosin)	20	5
	Saxostrea	Less tonic adductor	Smooth (Paramyosin)	20	5
	Squid	Mantle	Smooth (Helical)	2000	6
Arthropoda	Crayfish	Abdomen	Striated	3000	7
	Locust	Leg	Striated	3000	8
	Locust	Flight	Striated	3000	8
	Bee	Flight	Striated	5000	9
	Wasp	Flight	Striated	1210	10
	Housefly	Flight	Striated	5000	11
Vertebrates	Rabbit	Skeletal	Striated	2240	12
	Rabbit	Skeletal	Striated	484	10
	Rabbit	Skeletal	Striated	6620*b*	13
	Rabbit	Skeletal	Striated	1140*c*	14
	Pig	Uterus (pregnant)	Smooth (Classic)	170	15
	?	Uterus (pregnant)	Smooth (Classic)	1180*c*	16

a Activities are expressed in Qp units (Bailey, 1942), i.e. 22.4 × (μ moles of phosphorus split off from ATP in 1 hour by 1 mg. of protein).

b Value for myosin, which is usually lower than that for actomyosin.

c Values for heavy acto-meromyosin.

References: 1. Maruyama (1954a) 2 Tonomura *et al.* (1955) 3. Ruegg (1957) 4. Humphrey (1949a) 5. Humphrey (1949b) 6. Villafranca (1955) 7. Maruyama (1958c) 8. Gilmour and Calaby (1953) 9. Maruyama (1957) 10. Maruyama and Tonomura (1957) 11 Maruyama (1954b) 12. Greville and Needham (1955) 13. Mommaerts and Green (1954) 14. Mihályi and Szent-Gyorgyi (1953) 15. Needham and Cawkwell (1956) 16. Needham and Cawkwell (1958).

actomyosins of different muscles vary widely in their ATPase activity. This, however, can probably be attributed to the fact that the enzyme is extremely sensitive to the conditions under which it is isolated and studied. This is clearly brought out by the large differences in the values which have been reported for *Pecten* and even for rabbit skeletal muscles.

However, two significant results have recently been obtained. A comparison between the actomyosins of the tonic ("paramyosin") and striated parts of the adductor muscle of *Pecten* has shown that they both have about the same ATPase activity (Ruegg, 1957). And in vertebrate animals, it has been found that much the same activity is exhibited by preparations of heavy acto-meromyosin made from mammalian skeletal and uterine muscles (Mihályi and Szent-Györgyi, 1953; Needham and Cawkwell, 1958). In the case of *Pecten*, some earlier measurements (Tonomura *et al.*, 1955) also suggest that enzymatic activity is about the same in the two actomyosins, if allowance is made for the quantity of tropomyosin which very probably was present in the smooth muscle preparations; this tropomyosin has no ATPase activity (Ruegg, 1957).

Even though the inherent ATPase activity of myosin may be the same in all muscles, the rate at which the enzyme actually functions in life must be under the control of a variety of factors; about these, we have little information. Studies on glycerol-extracted fibers are of interest in this connection. Preparations of the tonic and phasic parts of the adductor muscle of *Anodonta* differ in their ATPase rates, and are less active than preparations of rabbit skeletal muscles (Ulbrecht and Ulbrecht, 1952). Such differences will partly depend on the quantity of actomyosin present in the muscle fibers. This is not known for *Anodonta* muscles, or, indeed, with any certainty for *any* invertebrate muscle.

4. *Tropomyosin*

Two kinds of tropomyosin are recognized in muscles (Bailey, 1956, 1957; Kominz *et al.*, 1957a, 1958). One of them, which here will be called "water-soluble tropomyosin" was discovered in vertebrate skeletal and cardiac muscles (Bailey, 1948) and has since been found in vertebrate smooth muscles and in several kinds of invertebrate muscles (Table IV). It occurs in rather small amounts; thus in rabbit skeletal muscles it accounts for about 8% of the total protein (Perry and Corsi, 1958).

TABLE IV

OCCURRENCE OF TROPOMYOSIN IN DIFFERENT MUSCLES.

Phylum	Genus	Muscle	Type	Type of tropomyosin[a]	Reference	Paramyosin[a]	Reference
Mollusca							
Lamellibranchs	Oyster	Tonic adductor	Smooth (Paramyosin)	W and I	1, 2	+	3, 4
	Oyster	Less tonic adductor	Smooth (Paramyosin)	W and I	1, 2	+	4
	Pinna	Tonic adductor	Smooth (Paramyosin)	W and I	1, 2	+	4
	Pinna	Less tonic adductor	Smooth (Paramyosin)	W and I	1, 2	+	4
	Pecten	Tonic adductor	Smooth (Paramyosin)	I	5	+	3, 4, 6, 7,
	Pecten	Striated adductor	Striated	(no I)	5	—	6, 7
	Venus	Tonic adductor	Smooth (Paramyosin)	A and B	8, 9	+	3, 4, 6, 7, 10, 11
	Venus	Less tonic adductor	Smooth (Paramyosin)			+	6, 7
	Mya	Adductor	Smooth (Mixed types)			+	3, 6, 7, 10
	Mytilus	Adductor	Smooth (Mixed types)			+	3, 4, 6, 7
Gastropods	*Buscyon*	Foot	Smooth (?)	A and B	8, 9		
	Helix	Foot	Smooth (Paramyosin)			+	4
	Viviparus	Foot	Smooth (Paramyosin)			+	3
Cephalopods	Squid	Mantle	Smooth (Helical)	W and B	8, 9, 12	—	4
	Sepia	Mantle	Smooth (Helical)	W	13, 14		
	Octopus	Arm	?	W and I	2		

TABLE IV (*continued*)

Phylum	Genus	Muscle	Type	Type of tropo-myosin[a]	Reference	Para myos-in[a]	Reference
Annelida	*Lumbricus*	Body wall	Smooth (Helical)	A	8, 9	+	15
	Arenicola	Body wall	Smooth (Helical)	A	8, 9		
Arthropoda	Lobster	Abdomen	Striated	B	8, 9		
	Scylla and Penaeus	Leg and thorax	Striated	W	13, 14, 16		
Vertebrates		Skeletal	Striated	W	17	—	6, 7
		Cardiac	Striated	W	17		
		Visceral	Smooth (Classic)	W (B)	13, 14, 16, 18	—	6, 7

[a] The letters A and B denote the two tropomyosins distinguished by Kominz *et al.* (1957a, 1958) and the letters I and W denote the *water-insoluble and water-soluble tropomyosins of* Bailey (1956, 1957). Data on the occurrence (+) or absence (—) of paramyosin structure, as observed in the electron microscope or in X-ray diffraction patterns, are also included.

References: 1. Bailey (1956) 2. Bailey (1957) 3. Hall *et al.* (1945) 4. H. and L. 5. Ruegg (1957) 6. Bear (1945) 7. Schmitt *et al.* (1947) 8. Kominz *et al.* (1957a) 9. Kominz *et al.* (1958) 10. Bear (1944) 11. Bear and Selby (1956) 12. Yoshimura (1955) 13. Jen and Tsao (1957) 14. Tsao *et al.* (1955) 15. Hanson and Lowy (1957) 16. Sheng and Tsao (1954) 17. Bailey (1948) 18. Kominz *et al.* (1957b).

The other kind of tropomyosin precipitates in water or weak salt solutions, and is therefore called "water-insoluble tropomyosin." It was discovered in the adductor muscles of *Pinna* and *Ostrea* (Bailey, 1956, 1957) and is found in very large amounts (as much as one-third of the total protein) here and in other smooth adductor muscles (Ruegg, 1957; H. and L.).

Although Kominz *et al.* (1957a, 1958) have used different criteria for classifying tropomyosins, they consider that their "tropomyosin A" corresponds to water-insoluble tropomyosin, and "tropomyosin B" to water-soluble tropomyosin.

Water-soluble tropomyosin (tropomyosin B) seems to be present in all kinds of muscles, including the tonic adductors of lamellibranch molluscs, which also contain the other tropomyosin. Water-insoluble tropomyosin (tropomyosin A) is more restricted in its distribution and, in general, the muscles which possess it are those which exhibit paramyosin structure. The best-known "paramyosin muscles" are those of the adductors of lamellibranch molluscs. But certain gastropod muscles also contain paramyosin filaments, and the equivalent muscles in the gastropod *Busycon* contain tropomyosin A.

In the cephalopod molluscs, it is very interesting to find that *Octopus* arm muscles have some water-insoluble tropomyosin. The structure of these muscles has not yet been examined in any detail, so that we do not know if they contain paramyosin. Water-insoluble tropomyosin has not been found in other cephalopods (*Loligo* and *Sepia*) where the mantle muscles were examined. Filaments with a structure which in some particulars resembles that of paramyosin have recently been observed in earthworm muscles, and here tropomyosin A is found.

Striated muscles do not show paramyosin structure, and no water-insoluble tropomyosin has been found in such muscles; but Kominz *et al.* (1957a) briefly refer to a rather similar tropomyosin which they have prepared from rabbit actomyosin.

No function has yet been established for either kind of tropomyosin. Tsao and his colleagues have suggested that these proteins have a "holding" function (Sheng and Tsao, 1954; Jen and Tsao, 1957). This idea was based on their finding that smooth muscles in vertebrates contain more tropomyosin than striated ones, but this difference no longer holds true (Perry and Corsi, 1958).

The presence of very large amounts of water-insoluble tropomyosin in the tonic muscles of molluscs suggests that this protein may in some

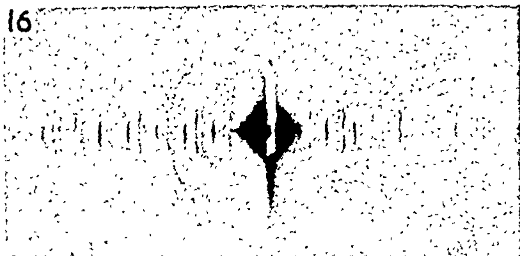
way be responsible for their ability to sustain tension for long periods of time. It is sometimes maintained that a "catch" mechanism operates in this type of muscle, and that once the muscle has contracted it can remain in that state *without any further energy consumption*, until it is stimulated to relax (Jordan, 1938). But it now seems more likely that tonus involves intermittent activity (Lowy, 1953) (p. 319). Nevertheless, these muscles are undoubtedly highly specialized for maintenance of tension, and although tropomyosin is not necessarily a "catch" protein (in the original sense of the term, as Hall *et al.* (1945) suggested *à propos* paramyosin), it is very reasonable to suppose that it is in some way concerned with the muscles' tonic function (Bailey, 1957).

Perhaps it would be possible to assign a function to the tropomyosin in these lamellibranch muscles if one knew where this protein and where actomyosin were situated in the muscle fibers, and what detailed structural changes occur on contraction. There is good evidence that the tropomyosin is situated in the paramyosin ribbons which seem to compose the filaments in the tonic fibers of lamellibranch adductors (Figs. 13–15). These ribbons have a fine structure which closely resembles that of the "crystals" precipitated from solutions of water-insoluble tropomyosin prepared from these muscles (Hanson *et al.*, 1957) (Figs. 17 and 18). Preliminary X-ray diffraction studies on this tropomyosin point to the same conclusion (Bailey, 1957). A different type of protein solution made from isolated paramyosin ribbons precipitates as banded "crystals" (Hodge, 1952; Locker and Schmitt, 1957) whose axial period is about the same as the length of the molecules of water-insoluble tropomyosin (Kay, 1958). The physical properties of such paramyosin solutions (Hodge, 1952; Locker and Schmitt, 1957) again indicate that paramyosin is equivalent to water-insoluble tropomyosin, which has recently been studied from this point of view (Kay, 1958).

The location of actomyosin in these tonic muscles is open to speculation (Elliott *et al.*, 1957). Its quantity is small, it has not yet been differentially extracted, and it precipitates in an amorphous form (Ruegg, 1957; H. and L.). Thus there is no evidence either for or against the idea that it may be situated together with tropomyosin in the paramyosin ribbons. (See Appendix)

B. Energy Sources

A great many investigations have been made on the phosphagen contents (arginine and creatine phosphates) of different muscles, and







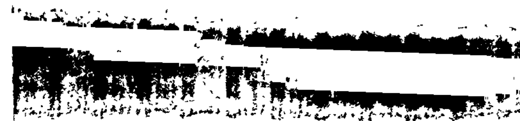


PLATE VI

some also on the quantities of ATP and ADP. With the reservation that unknown types of phosphagen may be present, such investigations give some idea of the total energy which may be available in a resting muscle for use in the initial period of activity following stimulation (Table V). There are interesting differences between cardiac, skeletal, and visceral muscles in vertebrates, and apparently also between the two parts of the oyster adductor. A few invertebrate muscles seem to contain extraordinarily large amount of phosphagen.

It would be more important, however, to know something about the *rate* at which the contractile elements can be supplied with sources of energy, for this must be one of the main factors which control the kind of activity a muscle carries out, for instance whether it functions rhythmically, or is specialized for tonic contraction. Unfortunately, nothing definite is known about such rates (see discussion in Pringle, 1957).

At least two phosphagens are quantitatively important in muscles —arginine and creatine phosphates—and others are also known (Thoai *et al.*, 1953; Rey, 1956). Several extensive comparative investigations have been carried out on invertebrate muscles to discover what kinds of phosphagen are present (see, for example, Baldwin and Yudkin, 1949), but as far as is known this has little bearing on the kind of function a muscle carries out, although it may be of evolutionary significance.

V. PHYSIOLOGY

A. Introduction

Most of the comparative physiological investigations that have been carried out on invertebrate muscles have been concerned mainly with

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PLATE VI. "Paramyosin smooth muscle." The opaque (tonic) part of the oyster adductor muscle.

FIG. 16. Low-angle X-ray diffraction pattern of a muscle dried at maximal body length. Paramyosin reflections (orders of 725 Å.) are visible on the meridian (here lying horizontally), in the direction of the fiber axis. (Taken by G. F. Elliott).

FIG. 17. Electron micrograph of a paramyosin element isolated from a muscle fiber by mechanical disintegration. Magnification: × 133,000 (Hanson *et al.*, 1957).

FIG 18 Electron micrograph of two "crystals," lying side by side, precipitated from a solution of water-insoluble tropomyosin. The one with a simple banding looks like a paramyosin element (Fig. 17); the other has a more complicated band pattern, which is, however, related to the simple pattern. Magnification: × 133,000 (Hanson *et al.*, 1957).

TABLE V

AMOUNTS OF PHOSPHAGEN, ATP, AND ADP IN DIFFERENT MUSCLES

Phylum	Genus	Muscle	Type	Phosphagen *a*	ATP *a*	ADP *a*	Ref.
Sipunculoidea	*Phascolosoma*	Body wall	Smooth (Classic)	3–22	—	—	1
	Sipunculus	Longitudinal	Smooth (Classic)	55	—	—	1
Mollusca	*Helix*	Pharynx retractor	Smooth (Classic)	—	1.7*b*	—	2
	Mytilus	Pedal retractor	Smooth (Paramyosin)	10	—	—	3
	Saxostrea	Tonic adductor	Smooth (Paramyosin)	3.6	1.2	3.0	4,5
	Saxostrea	Less tonic adductor	Smooth (Paramyosin)	4.5	2.7	5.4	4,5
Annelida	*Lumbricus*	Body wall	Smooth (Helical)	6.1	2.8	0.7	6
	Other genera	Body wall	Smooth (Helical)	2–30	—	—	1
	Other genera	Body wall	Smooth (Helical)	0.4–10	—	—	7
Echinodermata	*Marthasterias*	Tube feet	Smooth (Classic)	2	—	—	1
	Thyone	Body wall	Smooth (Classic)	5.7	—	—	1
	Echinus	Jaw	Smooth (Classic)	5.9	—	—	1
Chordata	*Balanoglossus*	Proboscis and collar	Smooth (Classic)	3.9	—	—	1
	Rabbit	Skeletal	Striated	32	9	0.9	8,9
	Rabbit	Cardiac	Striated	3	—	—	8
	Rabbit	Uterus (oestrus)	Smooth (Classic)	1.3	2.4	—	10
	Frog	Skeletal	Striated	9–18	3–5	0.3–0.9	2

a Expressed in μ moles per gram (wet weight) of tissue.
b One measurement only.

References: 1. Baldwin and Yudkin (1949) 2. Caldwell (1953) 3. Eggleton (1934) 4. Humphrey (1949a) 5. Humphrey (1949b) 6. Rey (1956) 7. Hobson and Rees (1955) 8. Ennor and Rosenberg (1952) 9. Bendall and Davey (1957) 10. Menkes and Csapo (1952).

the nervous control of contraction. This aspect of the subject has been reviewed in a recent monograph (Hoyle, 1957). Here the main object will be to examine certain mechanical properties of insect flight muscles and of molluscan smooth muscles, the only types of invertebrate muscle which have been thoroughly studied from this viewpoint. The reason why these muscles have attracted more attention than others is because they are capable of performances no vertebrate skeletal muscle can match. Thus the flight muscles of certain small midges (e.g. *Forcipomyia*) complete one contraction-relaxation cycle within 1 msec. (Sotavalta, 1947), and the smooth adductor of a lamellibranch mollusc like *Mytilus* maintains a large tension for several days without any signs of fatigue (Lowy, 1953). The question arises whether these specializations are developed from inherent properties common to all contractile systems, or whether they represent unique features. In recent attempts to supply an answer, results have been obtained which define more clearly the similarities and differences between various types of muscle, and so help toward a better understanding of the phenomenon of contractility.

B. Mechanical Properties of Resting Muscle

1. *Introduction*

The most interesting question concerning resting muscle is whether the contractile material is elastic or plastic (plastic in the sense that it will not support tension but has viscosity). In the case of vertebrate skeletal muscles, it has not yet been possible to obtain unequivocal information on this point, because there is an appreciable amount of connective tissue between the muscle fibers, and most of the resistance developed when the muscle is slowly stretched is believed to be due to such passive elasticity (Hill, 1952). However, in the flight muscles of insects, there is relatively little connective tissue, and Buchthal and Weis-Fogh (1956) have therefore chosen this type of striated muscle in an endeavor to study the mechanical properties of its resting contractile material.

Smooth muscles are altogether more difficult to investigate, for even after external denervation most of them show some tension due to spontaneous activity, and many of them are known to give an active response when stretched. Here the recent discovery of a drug (Twarog, 1954) which can selectively abolish the capacity for prolonged maintenance of tension in a "paramyosin" smooth muscle (of *Mytilus*) has ade it possible to obtain information, in that particular case, about the mechanical properties of the muscle at rest.



PLATE VII

For any consideration of the relation between tension and muscle length, it is necessary to choose a certain length for reference purposes. Here this will be taken as "maximal body length" (m.b.l.), which is defined as the greatest length the muscle can assume in the living animal. In the case of insect flight muscles, m.b.l. will correspond closely to "*in situ* length" (Buchthal and Weis-Fogh, 1956), because these muscles undergo length changes of only a few percent in the living animal. For the adductors of lamellibranch molluscs, m.b.l. is taken as the length of the muscle at the maximal gape of the shells ("reference length" of Abbott and Lowy, 1956a). In such muscles (and in all others that have hitherto been examined, see p. 307), it has been found that maximal active tension is developed at about m.b.l. (Abbott and Lowy, 1956a), and by analogy, in the case of the retractor muscles of *Mytilus* and *Helix*, the length at which they exert maximal active tension may be taken to correspond to about m.b.l.

2. *Insect Flight Muscle*

Buchthal and Weis-Fogh (1956) have investigated the relation between passive tension and extension in the flight muscles of the locust *Schistocerca*. They found that the empty sarcolemma tube breaks at a load which is only about 33% of that at which the intact fiber breaks. The corresponding figure for frog skeletal muscle is about 50% (Casella, 1951). Since the locust muscle develops a relatively large resting tension even below m.b.l. (Fig. 20, B) (about 10% of the maximal active tension compared with 1% in frog sartorius), Buchthal and Weis-Fogh suggested that in this muscle, the contractile material in the resting state has well-developed elastic properties. Although bumblebee (*Bombus*) flight muscle has not yet been fully investigated from this viewpoint, the fact that it also develops appreciable resting tension at lengths below m.b.l. (Fig. 20, A) (Boettiger, 1957a), indicates that here too, the contractile material in a resting state may possess appreciable elasticity.

Buchthal *et al.* (1957) have considered the possible functional sig-

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PLATE VII

FIG. 19. Striated muscle. *Calliphora* (blowfly) indirect flight muscle. Electron micrograph of a longitudinal section showing three fibrils and the large sarcosomes between them. Note the very short I bands. The pattern seen in the central fibril is due to the fact that the thick filaments pass in and out of the plane of the section. Magnification: × 17,300. (From an investigation by Dr. H. E. Huxley and one of the present authors—see Huxley and Hanson, 1957.)



PLATE VII

3. *Molluscan Smooth Muscle*

The behavior of the anterior byssal retractor of *Mytilus* (ABRM) and of the pharynx retractor of *Helix* has been studied by Abbott and Lowy (1958a), (Fig. 20, D, E). In the case of the *Mytilus* muscle, there is good evidence that part of the tension which appears on extension above m.b.l. is true resting tension, i.e. tension that is not due to spontaneous activity. The drug 5-hydroxytryptamine abolishes the ability of this muscle to maintain tension produced by such activity, without

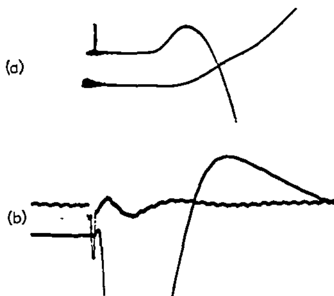


FIG. 21. Responses of squid mantle muscle to single shocks. Records read from left to right. (a) upper trace, transparency change (increase upwards); lower trace, development of tension (increase upwards). Duration of sweep, 50 msec. (b) upper trace, muscle action potential; lower trace, transparency change (increase upwards) recorded at lower amplification than in (a). Fifty cycles per second oscillation imposed on upper trace. (Note that in (b) the trace of the optical change at the end of the twitch crosses the baseline; this is an artifact due to recording with a condenser-coupled amplifier.) (Abbott and Lowy, 1956b.)

affecting its capacity to give large phasic responses and tensions (Twarog, 1954; Hoyle and Lowy, 1956). When a treated muscle is passively stretched, it still develops long-lasting resting tension (Abbott and Lowy, 1958a; Lowy and Millman, unpublished results).

C. FEATURES OF ACTIVE MUSCLE

1. *Optical Changes*

Apart from electrical responses, the events immediately following a stimulus have been studied intensively only in vertebrate skeletal muscle.

nificance of the large elastic forces within the locust muscle fiber. These authors observed that when the muscle is allowed to shorten from various lengths, more external twitch work is done when it starts at the greater lengths. But these differences largely disappear if it is assumed that a considerable part of the total work is derived from stored elastic energy (produced by passive stretch) rather than from the chemical energy liberated during contraction. In an isotonic twitch starting from m.b.l. (at 11°C.), the passive part of the work can be as much as half

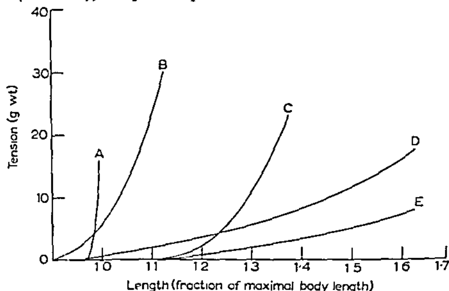


FIG. 20. Tension-length curves for muscles at rest. A. Bumblebee (*Bombus*) flight muscle (Boettiger, 1957a). B. Locust (*Schistocerca*) flight muscle, 11°C. (Weis-Fogh, 1956a). C. Frog sartorius muscle, 0°C. (Wilkie, 1956a). D. *Mytilus*, anterior byssal retractor muscle, 14°C. (Abbott and Lowy, 1958a). E. *Helix*, pharynx retractor muscle, 14°C. (Abbott and Lowy, 1956c).

the total work, and this stored energy can be converted into mechanical work at a rate which is of the same order of magnitude as the rate of working of the contractile system itself.

The flight muscles of the locust are known to undergo stretching by the inertia of the wing at the end of the up- or down-strokes (Jensen, 1956). The purpose of having a contractile material with well-developed elastic properties now becomes apparent; it could store some of the kinetic energy available at the end of the wing beat for use on the return stroke. In this connection, Buchthal *et al.* make the interesting point that a strong parallel elastic system might also be found in vertebrate skeletal muscles such as those responsible for respiratory movements in small fish, or for the wing movements of small birds.

cross-linked in much the same way as in striated muscle), it could account for the apparent absence of latency relaxation in these instances. Nothing can at present be said about the situation in "paramyosin" smooth muscles.

On the other hand, it also follows from these considerations that latency relaxation should be very pronounced in insect flight muscles, where elastic properties of the contractile material seem to be particularly well developed (see p. 303). But so far the early mechanical events associated with the onset of activity have not been specifically studied in insect muscles. However, Roeder (1953) has published a record of a twitch contraction in cockroach leg muscle from which it appears that latency relaxation may be present in that muscle, and could in fact be as high as 7×10^{-2} of twitch tension.

3. *The Tension—Length Relation*

It has been found that under isometric conditions, the active tension (total tension minus resting tension) developed by all the muscles that have been studied is a function of muscle length (Fig. 22). The total tension developed by glycerol-extracted fibers from rabbit psoas muscle, and from smooth lamellibranch muscles on treatment with ATP, also depends on length (Weber, 1956).

Inspection of the curves (Fig. 22) shows that in all the live muscles, active tension reaches a maximum just slightly above or below m.b.l., and decreases in a nearly linear manner on either side of that optimum. However, at about m.b.l. (for definition, see p. 303), the "paramyosin" type of smooth muscle (like ABRM and lamellibranch adductors) produces a much higher active tension than the other muscles. The highest value reported for frog skeletal muscle is about 3 kg. per square centimeter (Casella, 1951), and the highest figure established for any vertebrate muscle is 4 kg. per square centimeter (flexor of human elbow, Wilkie, 1954). In comparison, tensions up to 8 kg. per square centimeter have been obtained for the actively contracted ABRM at about m.b.l. (Abbott and Lowy, 1958b). The very high figures for the smooth adductors of lamellibranch molluscs, such as 35 kg. per square centimeter for *Venus*, which have sometimes been quoted from Marceau (1909) (Ritchie, 1928; Prosser, 1950; Hoyle, 1957), refer not to the active tension developed but to the force needed to stretch the contracted muscle.

In this connection, it may be noted that actively contracted ABRM

However, some relevant information has also been obtained by observing the optical and mechanical changes associated with the onset of activity in the remarkably transparent smooth mantle muscles of the squid (Abbott and Lowy, 1956b). Here an early small increase in transparency precedes the first sign of tension development, and coincides with the falling phase of the action potential (Fig. 21). This first small optical change is followed by a much larger *decrease* in transparency which lasts for about the duration of the twitch. The phenomena observed, and their time sequence, correspond to those that are known to take place in frog skeletal muscle (D. K. Hill, 1949), and in both types of muscle, the second optical change appears to be due to an increase in the amount of light that is scattered. But whereas at the peak of the twitch in the frog muscle, only 2 to 4% more light is scattered than at rest, the corresponding figure for the squid muscle is about 30%. The smooth squid muscle is therefore particularly well suited for studies of optical changes during contraction. As these manifestations of activity appear to be similar in both types of muscle, they may well reflect fundamental features of the contractile process.

2. *Latency Relaxation*

In striated vertebrate muscle stimulated isometrically at lengths where resting tension is present, a small drop in resting tension (latency relaxation) occurs at the same time as the small increase in transparency (D. K. Hill, 1949). Latency relaxation has not yet been detected in any of the smooth muscles that have been studied, (squid mantle muscle, *Helix* pharynx retractor, *Mytilus* ABRM); if it exists in the squid muscle, it must be less than 10^{-5} of the twitch tension (Abbott and Lowy, 1956b). In the frog sartorius muscle, latency relaxation is about 10^{-3} of twitch tension (Abbott and Ritchie, 1951).

A. F. Huxley (1957) has put forward the idea that latency relaxation in striated muscle is due to a lengthening of the thin (actin) filaments prior to cross-link formation. This implies that part of the resting tension may be due to elastic structures connecting the thin filaments longitudinally (as was suggested by Hanson and Huxley, 1955), or to these filaments themselves. It follows from arguments of this kind that the relatively small drop of tension in the thin filaments would be very difficult to detect when most of the resting tension is born by connective tissue outside the contractile material. If this were so in muscles like those of the snail or squid (where there are two kinds of myofilaments

persists for a considerable time after stimulation has ended (Lowy and Millman, 1959b).

As regards the other muscles, it is known that the locust flight muscle already begins to "give" when stretched while stimulated at about 10% above m.b.l. (Weis-Fogh, 1956a). The same might apply to the even more inextensible *Bombus* muscle. These muscles (or their attachments) evidently "give" more readily than frog sartorius.

Considering now the range of lengths over which all the various types of muscle function in the living animal, i.e., below m.b.l., the curves in Fig. 22 show that there are large differences. In some cases, these differences can be related to the manner in which the muscles function in the animals. The most striking contrast is between smooth muscles like the pharynx retractor of *Helix* (Abbott and Lowy, 1956c) or the body wall muscles of *Holothuria* (Hill, 1926) on the one hand, and the flight muscle of *Bombus* on the other. Thus while the *Helix* muscle can still produce appreciable active tension down to 35% of its m.b.l., the *Bombus* muscle no longer exerts any active tension below 88% (Fig. 22 A, E). It is well known that the snail muscle undergoes great changes in length as the animal's body is withdrawn from an extended condition right into the shell. In contrast, insect flight muscles work under nearly isometric conditions, the amount of shortening being only a few percent during flight (Weis-Fogh, 1956a; Boettiger 1957a).

Structural considerations may help to explain these findings. Thus at m.b.l., the length of the I bands is extremely small in insect flight muscles like those of *Bombus* (see review in Pringle, 1957). In fact, such a muscle at m.b.l. looks rather like a frog sartorius muscle which has been allowed to shorten to about 70% of its m.b.l. Below these lengths, when I bands have disappeared, neither muscle develops much active tension. Possibly this may have some connection with the fact that as soon as the I bands have been withdrawn into the A bands, the ends of the thick filaments seem to fold up where they are pushed against the Z lines, and form "contraction bands" (Volume I, Chapter VII). On the other hand, there are no Z lines in the smooth *Helix* muscle, and "contraction bands" are not seen at even very short lengths. In fact, unlimited sliding of the two sets of filaments relative to one another is theoretically possible (Hanson and Lowy, 1957).

4. *Shortening Speed and Load*

In all types of muscle that have been studied, it has been found from

and frog sartorius muscle behave alike in that the amount of tension redeveloped following a release decreases with the speed of release, and the amount of fall of tension after stretch decreases with speed of stretch (Abbott and Aubert, 1952; Abbott and Lowy, 1958b). When both types of muscle are stretched at speeds where "give" occurs (Katz, 1939), maintained tensions greater than the normal isometric value at the final length are obtained. But compared with frog sartorius

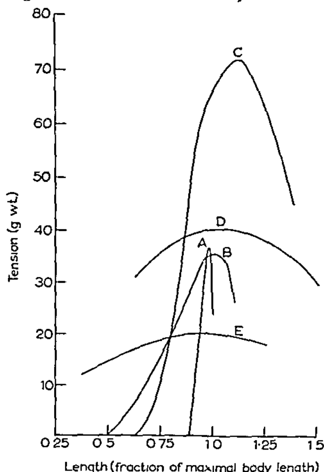


FIG. 22. Tension-length curves for active muscles. (Note: Tensions cannot be directly compared, as they are not given per unit cross section of muscle.) A. Bumblebee *(Bombus)* flight muscle (Boettiger, 1957a). B. Locust *(Schistocerca)* flight muscle, 11°C. (Weis-Fogh, 1956a). C. Frog sartorius muscle, 0°C. (Wilkie, 1956a). E. *Helix*, pharynx retractor muscle, 14°C. (Abbott and Lowy, 1956c). D. *Mytilus*, anterior byssal retractor muscle, 14°C. (Abbott and Lowy, 1958a).

muscle, the actively contracted ABRM shows a much greater resistance to stretch, and this is still further increased if instead of using repetitive shocks the muscle is stimulated with either direct current or acetylcholine; in the latter cases the increased resistance to stretch

ening, P_o the maximal tension the muscle can develop at zero speed, P the load on the muscle, a is a constant with the dimensions of a force, and b is a constant with the dimensions of a velocity. Thus the equation states that the velocity of shortening depends on the difference between the actual force on the muscle, and the maximal force it can develop. Hill (1938) established this relation on the basis of results obtained with frog sartorius muscle. Since then, tortoise, toad, ray, human arm (Wilkie, 1954), snail pharynx retractor, *Mytilus* ABRM (Abbott and Lowy, 1953), rat diaphragm muscle (Ritchie, 1954a), and mammalian uterine muscle (Csapo, 1955) have all been shown to obey the equation. So does locust flight muscle, if allowance is made for the presence of passive elastic forces by taking the load on the contractile material as the external load minus the resting load (p. 304) (Buchthal *et al.*, 1957). This has not yet been done for myogenic insect flight muscles (see p. 321), but the behavior of one such muscle (*Bombus*) during isotonic contractions strongly suggests that it, too, obeys the usual force-velocity relation (Boettiger, 1957b). In this respect, therefore, the only difference between the various types of muscle is one of time scale. Values for the maximal speed of shortening are assembled in Table VI, which shows that there is wide variation in speeds, the maximal shortening velocity for the more tonic part of the *Pinna* adductor being only about 1/100 that of frog sartorius muscle.

Even glycerol-extracted fibers from rabbit psoas muscle show the characteristic relation between load and velocity of shortening, during contraction induced by ATP (Ulbrecht *et al.*, 1954). It would be very interesting to know whether the rate of extra energy release (work plus heat above isometric level) during shortening is directly proportional to ($P - P_o$) in other muscles, as it is believed to be in the frog sartorius (Hill, 1938). But in this respect, the relation between thermal and mechanical events in other muscles has not yet been studied in detail.

Experiments with the smooth retractors of *Helix* and *Mytilus* indicate that as in frog muscle, the heat of shortening is proportional to the distance shortened, the values for the constant of proportionality (a, thermal) being of the same order of magnitude as those obtained for vertebrate skeletal muscles (Abbott and Lowy, 1958b). In passing, one interesting point may be noted. The values for the ratio a (thermal)/P_o in the smooth muscles are about 0.1, as compared with 0.25 for the frog sartorius, indicating that these smooth muscles may work more efficiently.

experiments under isotonic conditions, that as the load is increased the shortening velocity decreases. When the isotonic force is plotted against the initial velocity of shortening, a curve is obtained which shows that even at zero load, the muscle shortens with a definite limited velocity, and that the force is maximal under isometric conditions, i.e. at zero velocity of shortening (Fig. 23). The question arises how velocity is controlled. Hill (1938) has shown that in frog sartorius muscle, the extra heat liberated (above isometric level) for a given distance of

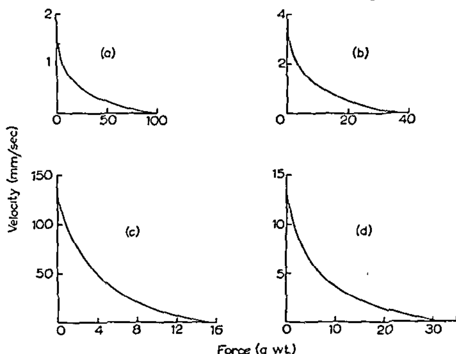


FIG. 23. Force-velocity curves. (a). *Mytilus* anterior byssal retractor muscle *Repetitive stimulation at 14°C. Shortening started from maximal body length* (Abbott and Lowy, 1958b). (b). *Helix*, pharynx retractor muscle; same conditions as for (a). (Abbott and Lowy, 1958b). (c). *Pecten*, cross-striated portion of adductor muscle; same conditions (as for (a). Abbott and Lowy, unpublished results). (d). Locust (*Schistocerca*). Curve calculated by Buchthal *et al.* (1957) from Hill's (1938) equation, using data obtained in experiments where the muscle was made to perform twitches at 11°C., starting from 91% of maximal body length.

shortening is independent of load. Thus a passive internal viscosity cannot be a decisive factor because it, and therefore the heat liberated, would increase at greater speeds. It is in fact believed (Hill, 1938) that the force on the muscle itself determines the rate of the chemical reactions, and in that way controls the velocity of shortening.

The force-velocity curve can be described by an equation given by Hill (1938): $v = (P_0 - P)\, b/(P + a)$ where v is the velocity of short-

developed by the contractile material, i.e., at the peak of an isometric twitch, or during the plateau of an isometric tetanus, the contractile material and the s.e.c. will be in equilibrium. If now a release is allowed at a speed greater than the muscle's maximal shortening velocity, the contractile material will not be capable of shortening quickly enough to prevent the undamped s.e.c. from shortening as well. Hence tension will fall, only to redevelop again as the contractile material finds time to shorten and restretch the s.e.c. From these considerations, it follows that when experimental conditions are so adjusted that tension just drops to zero, the extent of the release will be such that the s.e.c. can shorten completely. Thus a direct method is available for measuring the maximal amount of stretch of the s.e.c. under full tetanic tension.

There is also an indirect method which will give some estimate of the compliance (amount of stretch per unit applied force) of the s.e.c. It involves calculation of the shortening of the contractile material during the rise of tension in an isometric tetanus, and subtraction of this shortening from the total muscle shortening (Hill, 1949).

A knowledge of the compliance of the s.e.c. is necessary because it is one of the factors which determine the rate at which muscle develops tension. But before this is discussed, the concept of active state has to be considered.

Hill (1949) has defined the degree of activation (or intensity of active state) at any instant of time as the tension which the contractile material can sustain without either shortening or lengthening, and has produced evidence which indicates that active state reaches its full intensity very shortly after stimulation. According to Ritchie (1954b), activation in the frog sartorius muscle (at 0°C.) already begins to decay 35 msec. after the stimulus, and remains at full intensity for only about 25 msec. Now the fully activated contractile material has to stretch an undamped s.e.c. by a definite amount before external tension can be manifested, and because the shortening velocity of the contractile material will decrease as tension in the s.e.c. increases, the rate of rise of external tension is comparatively slow (Hill, 1949). Hence, in a twitch, there may not be time for the (fully activated) contractile material to shorten enough to stretch the s.e.c. to its full extent before relaxation sets in, and the maximal tension will therefore be less than in a tetanus. From such arguments, it follows that other factors being equal (e.g. shortening velocity, series-elastic compliance, the longer the duration of the active state, the closer will the ratio, tetanic tension/twitch

TABLE VI

AVERAGE VALUES FOR THE MAXIMAL SPEED OF SHORTENING (V_o) IN DIFFERENT MUSCLES ACTIVATED AT MAXIMAL BODY LENGTH

Muscle	Temperature (°C.)	V_o (muscle lengths per second)	Reference
Vertebrates			
Rat diaphragm	37	11	1
Frog sartorius	22	10	2
Human elbow flexor	37	6	3
Ray coraco-mandibularis	0	2	3
Toad sartorius	10	1.5	4
Tortoise iliofibularis	20	0.4	4
Rabbit uterus	37	0.2	5
Invertebrates			
Locust flight	30	13	6
Pecten striated adductor	14	3	7
Octopus funnel retractor	18	2.4	8
Mytilus pedal retractor	14	0.7	8
Mytilus anterior byssal retractor	18	0.3	8
Helix pharynx retractor	14	0.2	9
Pinna tonic (opaque) adductor	18	0.1	8

References: 1. Ritchie (1954a) 2. Hill (1938) 3. Wilkie (1954) 4. Abbott and Lowy (1957) 5. Csapo (1955) 6. Weis-Fogh (unpublished results) 7. Abbott and Lowy (unpublished results) 8. Lowy and Millman (unpublished results) 9. Abbott and Lowy (1953).

5. *The Series-Elastic Component and Active State*

The presence of a series elasticity in muscle has been inferred from the following experimental observation: when isometrically activated muscle is allowed to shorten rapidly by a few percent of its length, tension falls to zero, and then rises again to the normal isometric level characteristic of the shorter length (Gasser and Hill, 1924). This quick-release-recovery effect has been observed in all types of striated and smooth muscles hitherto studied, as well as in glycerol-extracted fibers and actomyosin threads (Weber, 1956). For living muscle, it has been interpreted in terms of a model which contains a virtually undamped elastic component in series with the contractile material (A. V. Hill, 1949). On that model, during isometric contraction, the contractile material shortens to some extent and stretches the series-elastic component (s.e.c.). The shortening of the contractile material will stop when the force due to the s.e.c. becomes equal to the maximal tension

material and the undamped s.e.c. are in equilibrium, the tension exerted by the contractile material will represent the degree of activation remaining. Ritchie (1954c) has developed a method for following the decay of the active state. He released frog sartorius muscle by a small amount at various times after the start of an isometric twitch, and recorded the tension redeveloped at the shorter length. It follows from what has been said above that the peak of each curve (redevelopment of tension followed by decay) lies along the active state curve (Fig. 24, *a*). The redevelopment of tension corresponds to the phenomenon of tension recovery after a quick release (Gasser and Hill, 1924).

When Ritchie's experiment was performed with the smooth pedal retractor muscle of *Mytilus*, there was found to be a significant difference in the extent to which tension outlasts active state (Fig. 24, *b*) (Abbott and Lowy, 1958b). This is even more pronounced in the ABRM where the decay of tension can be two orders of magnitude slower than that of active state; and this also applies when active state is prolonged by stimulating the muscle for several seconds either with direct current or with acetylcholine (Lowy and Millman, 1959a, b). The tension present after active state has decayed to zero may be called passive tension.

Some information is also available about the decay of active state in two insect muscles. Ritchie (1954c) found in experiments with frog sartorius, that the shorter the muscle, the more rapid the decay of active state. Accordingly, Buchthal *et al.* (1957) have constructed curves from twitch maxima at various muscle lengths, which they consider to indicate the time course of decay of active state in locust flight muscle (Fig, 24, *d*). Pringle (1954; 1957) has published records of twitches and an "active state curve" for the tymbal muscle of a cicada (Fig. 24, *f*), obtained by plotting the reciprocal of the time intervals between "IN" clicks made by that muscle in response to a single shock (see p. 322).

Inspection of all the curves in Fig. 24, *c*—*f* shows that (1) in every instance tension outlasts active state, (2) the muscles appear to differ in the extent to which this happens, and (3) the ratio, tetanus tension/twitch tension, varies.

For striated muscle, (1) has been explained in terms of a mechanism based on the sliding filament model, which assumes that the time constant for breaking cross-links is longer than the time constant for their

tension, approach unity. Hill (1949) believes that the contractile material is maximally activated even in the relatively short duration of a twitch because by extending frog sartorius muscle rapidly by about 10% of its length, very shortly after the stimulus, full tetanic tension appears, and this Hill assumes to be due entirely to the removal of the effect of the s.e.c.

After stimulation has ended, active state decays with time, and on Hill's definition, at the peak of an isometric twitch, when the contractile

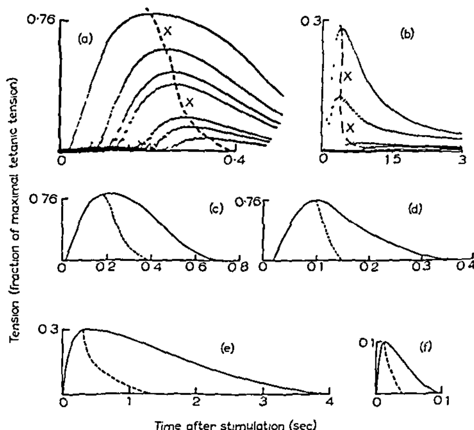


FIG 24 Active state.

(a) and (b) Active state curves obtained from experimental tension-time curves. Curves (x) drawn through peaks of tension-time curves show decline in intensity of active state. (a). Records of tension development in frog sartorius muscle at 0°C, followin The stimulus coincid ; (b). Same as (a) for .

(c) — (f) Isometric twitches and active state curves. Active state curves (interrupted) obtained as described in text. (c). Frog sartorius muscle. 0°C. (Ritchie and Wilkie, 1955). (d). Locust *(Schistocerca)* flight muscle, 11°C. (Buchthal *et al*, 1957). (e). *Mytilus*, pedal retractor muscle, 14°C. (Abbott and Lowy, 1958b); (f) Cicada *(Platypleura)* tymbal muscle, 30°C. (Pringle, 1954; 1957).

material and the undamped s.e.c. are in equilibrium, the tension exerted by the contractile material will represent the degree of activation remaining. Ritchie (1954c) has developed a method for following the decay of the active state. He released frog sartorius muscle by a small amount at various times after the start of an isometric twitch, and recorded the tension redeveloped at the shorter length. It follows from what has been said above that the peak of each curve (redevelopment of tension followed by decay) lies along the active state curve (Fig. 24, *a*). The redevelopment of tension corresponds to the phenomenon of tension recovery after a quick release (Gasser and Hill, 1924).

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For striated muscle, (1) has been explained in terms of a mechanism based on the sliding filament model, which assumes that the time constant for breaking cross-links is longer than the time constant for their

formation (A. F. Huxley, 1957). But the significance of (1) and (2) probably cannot be assessed in these terms only, because recent experiments with vertebrate skeletal muscles have shown that the greater the extent of the quick release (see Ritchie's method), the more rapid is the decay of the active state. Evidently, active state is not merely a function of time (Goodall, 1958).

The variations in the tetanus/twitch ratio should be explicable in terms of differences between (a) rates of decay of active state, (b) maximal shortening velocity, and (c) compliance of the s.e.c. Taking the case of locust flight muscle, Buchthal *et al.* (1957) have shown that its tetanus/twitch ratio is 2, compared with 4.6 for frog sartorius (both muscles stimulated at 25°C.). The maximal shortening velocity of the two muscles does not differ greatly (see Table VI). But the compliance of the s.e.c. may be less in the locust muscle. Using Hill's (1949) indirect method (see p. 313), Buchthal *et al.* (1957) calculate that under full tetanic tension, the maximal extension of the s.e.c. is 1 to 2% of the muscle's length, and quote the corresponding figure of 10%, calculated by Hill (1949) for the frog muscle. The direct method, however, gives values of 3 to 4% for the frog muscle (Hill, 1950), and it is therefore conceivable that the correct value for the locust muscle will prove to be rather less than 1%. It may be noted that for the locust muscle, most of the series elasticity may reside within the fiber, because the cross-striations extend to within a few microns of its attachment to the cuticle (Buchthal and Weis-Fogh, 1956).

Recently, an isotonic release technique (Wilkie, 1956b) has given results which indicate that a 2% shortening will give the series-elastic effect in frog sartorius muscle (Jewell and Wilkie, 1958). Jewell and Wilkie estimate that about half the series elasticity resides in tendon material. It would appear therefore that in both the frog and locust muscle, the compliance of the series elasticity within the fiber is about the same.

But the total compliance of the s.e.c. is still different in the two types of muscle. Nevertheless, this does not necessarily account fully for the higher twitch tension observed in the locust muscle, because Buchthal *et al.* (1957) found that the rate of decay of active state is relatively faster in the locust than in the frog muscle. On the other hand, a very short duration of active state may be the critical factor in the case of the cicada tymbal muscle, (see p. 322), where the tetanus/twitch ratio is about 10 (see Fig 24, *f*). In experiments with this muscle, Pringle

(1954) obtained a figure of 1.5% for the extent of rapid shortening necessary to produce a very substantial drop of isometric tension. Apparently, the compliance of the s.e.c. does not differ appreciably from that of the locust muscle. Although the active state curve for the cicada muscle was not obtained in the same way as that for the locust muscle, it does indicate the possibility that in the cicada muscle, maximal activation may last for a much shorter time.

The outstanding feature as regards *Mytilus* muscle is the extent to which tension outlasts active state. In the ABRM the tetanus/twitch ratio is high (about 8; see Abbott and Lowy, 1953) whereas the series compliance is of the same magnitude as in striated muscles, i.e. a 4–6% shortening will give the series-elastic effect (Abbott and Lowy, unpublished results). Considering that in this muscle the rate of rise of tension following a quick release is appreciably greater than that of the initial tension rise it would appear that here maximal activation is attained by some process of recruitment (Lowy and Millman, 1959a).

6. *The Effect of a Quick Release on Active State*

Consideration of the quick-release-recovery phenomenon in terms of the sliding filament model suggests a possible explanation of the effect of a quick release on active state. According to A. V. Hill's (1949) interpretation (see p. 312), provided the release is more rapid than the maximal shortening velocity of the contractile material, the observed momentary loss in tension is due entirely to shortening of the s.e.c. However, A. F. Huxley (quoted by H. E. Huxley, 1959) has suggested that the phenomenon might be accounted for if it is assumed that during the quick release, the contractile material does in fact shorten very rapidly by a very small amount, and that a certain time has to pass before it can again start a new cycle of operation. As H. E. Huxley (1959) points out, this would account for Hill's (1953) observation that the heat output during a quick release of isometrically contracted frog sartorius muscle is 3 to 4 times greater than the normal heat of shortening over the same distance. H. E. Huxley explains this as follows. During the plateau of an isometric contraction, a relatively large number of cross-links is in action. When the muscle has been released, many cross-links will still be at the beginning of a cycle of operation which they must complete, although the muscle now shortens against a much smaller load than they would normally sustain. The extra energy thus dissipated by these cross-links could represent the heat produced above the normal level of shortening heat.

On the basis of this kind of argument, the effect of a quick release on active state could be explained as follows. When isometrically contracted muscle is allowed to shorten to just the extent which reduces tension to zero, movement of the filaments relative to one another is so small that cross-links do not detach. In this case, the subsequent speed of redevelopment of tension would be expected to be more rapid than the speed of the initial tension development at the beginning of stimulation when cross-links have to be formed. However, as the extent of the release is increased, the cross-links would eventually detach. They would then have to reform, and this could be reflected as a decrease in the speed

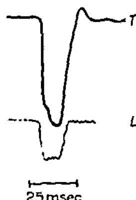


FIG. 25. Bumble-bee (*Bombus*) flight muscle. Release and stretch of isometrically tetanized muscle by 1 to 2% of its initial length. L, length; T, tension (Boettiger, 1957b).

of redevelopment of tension, so that a quick stretch imposed shortly after such a release would reveal the presence of relatively less tension.

Perhaps the effect of a quick release is more dramatic in insect flight muscle, because as there is very little tendon material at the ends of the fibers, even a relatively small shortening results in detachment of cross-links. Thus when the longitudinal flight muscle of *Bombus* is allowed to shorten rapidly by 1 to 2% of its length, only 30 to 50% of the original tension is recovered immediately when the muscle is stretched again to its initial length (Boettiger and Furshpan, 1954a, b). Pringle (1954) observed a similar phenomenon in the tymbal muscle of a certain cicada, and suggested that the contractile material is deactivated by the quick release, and that such an effect might be found in all striated muscles. The demonstration by Goodall (1958) that active state in turtle skeletal muscles is affected by a quick release (see p. 316) supports Pringle's hypothesis.

At this stage a phenomenon could conveniently be discussed which

has so far been observed in *Bombus* muscle only. Quick-release experiments have shown that the initial drop in tension is followed by another, more gradual fall in tension after the length change is over (Fig. 25) (Boettiger and Furshpan, 1954a, b). On the above explanation, it can be argued that cross-links may have become detached during the quick release. If the thin filaments were to lengthen prior to re-formation of cross-links, tension would drop slightly without an accompanying change in muscle length (elastic elements connecting the thin filaments across the H zone would take up the slack). A similar sequence of events has been proposed to account for latency relaxation (see p. 306). It has already been pointed out that because the passive elastic properties of the contractile material in insect flight muscles seem to be particularly well developed, these muscles should show a pronounced latency relaxation (p. 307). The explanation for the phenomenon described here could be tested by isometrically tetanizing *Bombus* muscle at various lengths, allowing the same extent of rapid shortening, and studying the second phase of the tension decline. It would also be interesting to know whether such a phenomenon occurs after rapid shortening in any other muscle and to observe the effects of allowing active *Bombus* muscle to shorten rapidly by less than 1% of its length.

D. The Prolonged Maintenance of Tension in Molluscan Smooth Muscle

We shall now examine more closely the behavior of two muscles which differ greatly in structure and function, namely the very rapidly oscillating flight muscles of insects, and the particularly slow molluscan "paramyosin" smooth muscles. The latter can maintain a high level of tension for long periods without signs of fatigue, as activation appears to be necessary only at very infrequent intervals (Lowy, 1953). This is probably because in such muscles tension (passive tension) persists long after active state has decayed to zero, (see p. 315). The explanation put forward here does not accept the 'catch mechanism' hypothesis which holds that these muscles can maintain tension indefinitely without any expenditure of energy (Jordan, 1938; Twarog, 1954; Barnes, 1955). It has been found (Abbott and Lowy, 1958,b) that in the absence of further activation tension decays to zero, and that a steady level of tension can only be maintained by periodic re-excitation of the muscle, i.e. by a tetanic mechanism.

The striking difference between the behavior of these "paramyosin"

On the basis of this kind of argument, the effect of a quick release on active state could be explained as follows. When isometrically contracted muscle is allowed to shorten to just the extent which reduces tension to zero, movement of the filaments relative to one another is so small that cross-links do not detach. In this case, the subsequent speed of redevelopment of tension would be expected to be more rapid than the speed of the initial tension development at the beginning of stimulation when cross-links have to be formed. However, as the extent of the release is increased, the cross-links would eventually detach. They would then have to reform, and this could be reflected as a decrease in the speed

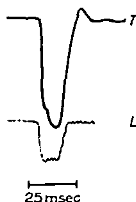


Fig. 25. Bumble-bee (*Bombus*) flight muscle. Release and stretch of isometrically tetanized muscle by 1 to 2% of its initial length. L, length; T, tension (Boettiger, 1957b).

of redevelopment of tension, so that a quick stretch imposed shortly after such a release would reveal the presence of relatively less tension.

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E. Myogenic Rhythmic Contraction in Insect Flight Muscle

It has been demonstrated recently that under certain conditions, maintained auto-oscillation (myogenic rhythmic contraction) can be induced in glycerol-extracted mammalian skeletal muscle fibers by treatment with ATP (Goodall, 1958; Lorand and Moos, 1956). The capacity for myogenic rhythmic contraction is recognized to be particularly well-developed in rapidly oscillating insect flight muscles, where it has been intensively studied during the past few years.

A large insect like the locust has a comparatively low wing beat frequency of about 18 beats/sec. (Weis-Fogh, 1956b). In smaller insects like flies and bees, the frequency ranges from 100 to 300 beats per second, and in some midges a frequency of 1000 beats per second has been reported (Sotavalta, 1947). It has been found that in the flight muscles of the larger insects, there exists, as in frog sartorius, a 1:1 relation between electrical and mechanical responses (Roeder, 1951), whereas in insects with a wing beat frequency greater than about 100 beats per second, the flight muscles possess a rhythmic myogenic system, and the electrical responses in the muscle are not related to wing movements (Pringle, 1949). Thus in the blowfly *Calliphora*, the muscle action potentials (recorded with external electrodes) were found to occur at a frequency of about 3 per second, for a wing beat frequency of 120 beats per second (Pringle, 1949). This discovery was later fully confirmed in experiments with intracellular electrodes (Boettiger and McCann, 1953).

Another unusual feature of rapidly oscillating insect flight muscles has been known since it was first described by de Geer in 1776, namely that complete or partial removal of the wing results in an increase of the oscillation frequency of the flight muscles. Working with *Calliphora*, Pringle (1949) was able to show that this is not a reflex due to sense organs, in muscle or wing, but that it is a direct influence of loading on the frequency of the myogenic rhythm.

The myogenic flight muscles (Fig. 19) are distinguished by having much larger and many more sarcosomes than ordinary muscles (Watanabe and Williams, 1951). Because of this, the fibrils are well separated from each other, and can easily be isolated from the fibers. The fibrils are also larger than those found in the 1:1 type of flight muscle. For these reasons, a histological distinction has been made, the myogenic muscles being referred to as "fibrillar," and the 1:1 type as "non-fibrillar" (see review by Pringle, 1957). To avoid misunderstanding, it must be pointed out, however, that both types of muscle really possess fibrils, i. e. special groups of myo-

muscles and that of frog sartorius is illustrated in Fig. 26, which shows that while peak twitch tension is reached in about 200 msec. both in the "paramyosin" adductor of *Pinna* (at 15°C.) and in the frog muscle (at 0°C.), tension in the smooth muscle decays to half-value within 1.5 sec., compared with about 180 msec. in the frog muscle.

Another important feature of "paramyosin" smooth muscles may be their relatively low maintenance heat rate. Thus about 20 sec. after the beginning of repetitive stimulation, the maintenance heat rate for the ABRM of *Mytilus* under isometric conditions at 14°C. is only about 0.1 mcal./g./sec. (Abbott and Lowy, 1955), while the corresponding figure for frog sartorius at 0°C. is 1.5 (Abbott, 1951). It appears that during activation, the rate of energy expenditure in the ABRM is much

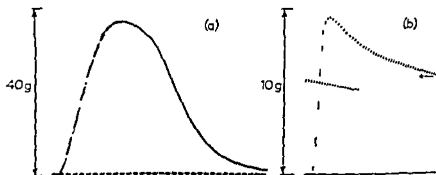


FIG. 26. Isometric twitches at maximal body length. Shock at origin of trace. Time marks, 20 msec. (a). Frog sartorius muscle, 0°C. (Ritchie and Wilkie, 1955). (b). *Pinna*, less tonic (translucent) portion of posterior adductor muscle, 15°C. Tension followed for $1\frac{1}{2}$ sweeps (Abott and Lowy, 1956a).

smaller. Now supposing that as in frog muscle (Maréchal and Aubert, 1958), there occurred in the smooth muscle a progressive decrease in the maintenance heat rate, then it is conceivable that after a certain time, the activation required to maintain a given level of tension would be associated with a comparatively minute energy expenditure.

There is a third factor which might help to explain the performance of the lamellibranch muscles. By recording muscle action potentials in the smooth adductor of an intact animal (*Mytilus*) over a period of several days, it has been found that the same high level of tension can be maintained at a progressively decreasing frequency of electrical activity, thus indicating that the amount of reactivation required to maintain the tension seems to diminish with time (Lowy, 1953).

of a 1:1 type of muscle, like the dorsal flight muscle of the locust *Schistocerca*, there is a skeletal click mechanism which could "assist" relaxation (Weis-Fogh, quoted by Pringle, 1957).

In view of these findings, the operation of a quick-release (and maybe of a quick-stretch) mechanism, modulating the degree of activation in rapidly oscillating insect flight muscles, seemed a reasonable possibility. It was then discovered that (as in glycerol-extracted mammalian skeletal muscle fibers) spontaneous and nearly sinusoidal oscillations could be obtained in the longitudinal flight muscles of *Bombus*, working under an inertial load, in the complete absence of a click mechanism (Boettiger, 1957a).

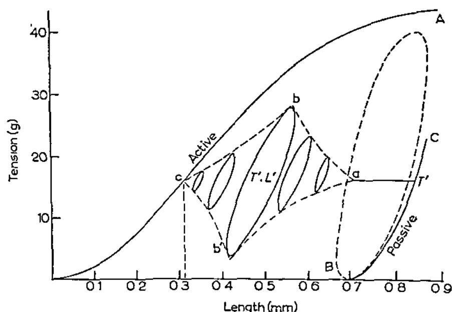


FIG. 27. Behavior of bumblebee (*Bombus*) flight muscle during isotonic tetanic stimulation with a weight (Boettiger, 1957b). For explanation, see text.

The *Bombus* preparation was mounted so that one electro-mechanical transducer recorded changes in length, and another changes in tension, on the vertical and horizontal plates respectively of a cathode ray tube. This made possible the direct tracing of relations between length and tension. As has already been pointed out (see p. 303 and p. 307), these relations are not essentially different from those observed in other muscles. The special properties of the insect muscle are only revealed when it is stimulated under isotonic conditions with a load. When loaded to tension T', it shortens to the length indicated at *a* in Fig. 27 and then begins to oscillate spontaneously with increasing amplitude,

filaments, separated from each other by non-fibrillar sarcoplasm. There is another point which could conveniently be clarified here. Tiegs (1955) reached the conclusion that in "fibrillar" muscles, the fibrils ("sarcostyles") are composite. But electron micrographs of transverse sections through such fibrils (Hodge *et al.*, 1954; Hodge, 1955) show that normally they are not subdivided. Under abnormal conditions, the submicroscopic filaments probably become separated from each other into small groups, visible in the light microscope.

Pringle (1954) has elucidated the cycle of mechanical events in the rapidly oscillating sound producing "tymbal" muscles of the cicadas *Platypleura capitata* and *P. octoguttata*. On excitation, this type of muscle contracts almost isometrically (like insect flight muscle, see p. 309) against the elastic resistance of the tymbal cuticle, until at a certain critical tension, controlled by a tonic tensor muscle, the tymbal buckles like the lid of a tin and emits a pulse of sound. Pringle was able to demonstrate in experiments with a nerve-muscle preparation that at 30°C., a single motor nerve impulse evokes 4 sound pulses, and that stimulation at 50 shocks per second sets up a myogenic rhythm at about 320 oscillations per second. He suggested that the very high frequency of the contraction-relaxation cycle is due to the sudden release of tension at the "IN" click which "assists" relaxation by deactivating the muscle (see p. 318), so that it can be pulled out again by the elasticity of the tymbal cuticle. As long as the requisite number of nerve impulses reach the muscle fiber, when the tymbal clicks to the "OUT" position, tension in the muscle will rise again until the tymbal once more clicks "IN." Thus when the muscle is attached to the tymbal, 4 clicks can occur during 40 msec., which appears to be the duration of "activity" set up by one motor nerve impulse. Pringle describes this system as one in which work is done through a cycle of activation and deactivation, and not as in frog sartorius by excitation and recovery. In his hypothesis, Pringle did not exclude the possibility of a direct effect of stretching on the active state of the contractile material, a condition which Boettiger (1957b) considers to be essential for the maintenance of the cycle.

Studies on the articulation of the dipteran wing and experiments with partially anesthetised animals (Boettiger and Furshpan, 1952) showed that in this case, too, the relaxation of the rapidly-oscillating flight muscles is "assisted" by a click action, the energy being produced by the natural elasticity of the thorax itself. The part played by the well-developed passive elastic properties of the contractile material has already been noted (see p. 304). It now appears that even in the case

(3). The velocity with which the contractile material in muscle (or glycerol-extracted fibers) shortens, decreases with increasing load in a hyperbolic manner. This relation can be described by Hill's (1938) characteristic equation.

In frog sartorius muscle, it has been shown that the contractile material can release varying amounts of total energy for a constant change of muscle length, and it would appear that the tension the muscle is generating controls the rates of the energy producing reactions. It is not yet known if this applies to other types of muscle.

(4). The rates of the energy producing reactions, and hence the degree of activation (here defined as the active tension which the contractile material can exert at any instant), are affected by rapid shortening (quick-release) and possibly by rapid stretching as well.

(5). When isometrically contracting active muscle is allowed to shorten rapidly by a few percent of its length, tension falls to zero and then rises again to the value characteristic of the shorter length (quick-release-recovery phenomenon). This phenomenon can be interpreted as being due to series-elasticity. The compliance of this elasticity, the rate of decay of active state, and the intrinsic shortening velocity of the contractile material will determine the rate at which external tension develops at the beginning of stimulation.

(6). The contractile material which possesses the properties so far enumerated is constructed from large protein molecules. According to X-ray diffraction data, many of these molecules are fibrous and lie with their polypeptide chains parallel to the direction of shortening. These chains have an α-helical configuration.

It seems that in all types of muscle, the protein molecules which constitute the contractile apparatus are grouped into filaments which are thick enough to be seen in electron micrographs.

(7). One of the most important general questions, to which there is at present no definite answer, is whether or not the individual filaments in the contractile apparatus are themselves ever contractile. There is no convincing evidence that they are. On the contrary, in striated muscles contraction appears to be brought about by movements of two kinds of filaments relative to one another. Such a "sliding filament" mechanism probably also operates in certain smooth muscles which, like striated ones, are constructed from two kinds of cross-linked filaments.

The basis of the contractile mechanism in vertebrate striated muscles

until a steady state 'bb' is reached. (Unfortunately, no figures are given by Boettiger (1957a, b) for the maximal frequency of these oscillations. In the animal, the muscle can perform up to 100 oscillations per second.) From the counterclockwise direction of movement of the spot on the cathode ray tube, it was possible to deduce that during these oscillations, the muscle is doing work, which in the animal would be represented by the movements of the wings through the air.

Boettiger (1957b) has considered the requirements for the maintenance of sinusoidal oscillatory movements in a self-exciting system. On his model, motion is due to an internal force component, which changes its sign and magnitude throughout the cycle, in such a manner that it "assists" both shortening and lengthening. This component is supposed to arise as the result of a shift in the phase relations between tension and length. Boettiger states that such a shift can actually be observed when an excited *Bombus* muscle is made to shorten and lengthen at various frequencies by an external mechanical system.

Finally, there is another observation which has to be taken into account when considering how these muscles function. Hanson (1956a, b) found that myofibrils isolated from the myogenic flight muscles of *Calliphora* could be made to elongate and shorten, alternately, under the microscope. It was shown that elongation was "active" in the sense that it was effected by the sarcomeres and was not caused by a pull exerted by other contracting sarcomeres.

VI. CONCLUSIONS

A. Features of the Contractile Apparatus which are Probably Common to all Types of Muscle

(1). The tension exerted by active muscle (or by glycerol-extracted fibers) is a function of length. Normally (see 2), maximal active tension is attained at about the greatest length the muscle can assume in the living animal, and decreases in a nearly linear manner on either side of this maximum. Different types of muscle (or glycerol-extracted fibers) develop maximal tensions in the range from 1 to 10 kg. per square centimeter of muscle cross section.

(2). When active muscle is stretched at speeds where "give" occurs it becomes capable of maintaining a tension which is higher than which it would normally exert at the longer length. Thus tensions greater than the normal maximum can be attained.

of 1000 cycles per second. Structurally, they are notable for their numerous large sarcosomes, which enable rhythmic activity to be maintained for long periods without incurring an oxygen debt. An ability to oscillate seems to be inherent in the muscles. In the intact animal, a skeletal "click" mechanism may control the rhythm. Flight muscles are remarkably responsive to rapid length changes, possibly because there is very little tendon material at the ends of the fibers. Thus a quick release of isometrically contracted muscle results in considerable deactivation, so that rapid re-extension of the muscle (due to the elasticity of the skeleton) is facilitated.

Passive elastic properties of the contractile material are much better developed than in other striated muscles and play an important part in the dynamics of flight.

C. The Significance of Helically Arranged Smooth Myofibrils

Smooth muscles in which the myofibrils are helically arranged very often contract and relax more rapidly than other types of smooth muscle. This apparent correlation is probably misleading, for such muscles are also notable for their well-differentiated fibrils with closely and often regularly packed filaments, and for their highly organized sarcoplasmic reticulum. These features may be of critical importance in determining the maximal speed at which the fiber can contract and can be brought into action.

The helical arrangement of the fibrils may serve a different purpose. It could extend the range of muscle lengths over which tension is exerted, for as Marceau (1909) pointed out, a given shortening of helical fibrils (as compared with strictly longitudinal ones) will result in a greater shortening of the muscle fiber. This may indeed be the function of "helical smooth muscles" in some animals. On the other hand, it is well known that certain "classic smooth muscles," such as the body wall retractors of holothurians and the pharynx retractor of the snail *Helix*, can develop tension over an exceptionally wide range of muscle lengths.

D. The Meaning of Striation

Filaments of two kinds, lying alongside each other and cross-linked together, are present in many muscles. In some, the two kinds of filaments are segregated into regular transverse arrays which alternate

is that those filaments which contain actin move relative to those that contain myosin. There is reason to believe that these movements are in some way mediated by ATP. *In vitro*, the interactions of actin, myosin, and ATP reflect their probable behavior *in vivo*. Hence it may be significant that muscles of every type contain actomyosin, and that the actomyosins prepared from different muscles all react with ATP in the same manner.

(8). No differences have been established between the ATPase activities, *in vitro*, of the actomyosins prepared from different types of muscle.

(9). The contractile system contains tropomyosin, as well as actomyosin. Of the two kinds of tropomyosin which are at present recognized, one is common to all types of muscle, where it is found in rather small amounts. Its function is unknown.

These then are the known properties of muscle which will have to be accounted for in any general theory of contractility. Such a theory cannot yet be attempted.

B. Examples of Specializations in Invertebrate Muscles

1. *Molluscan "Paramyosin Smooth Muscle"*

These muscles have a remarkable structure (see also Appendix), their filaments apparently being constructed from ribbon-shaped paramyosin elements which contain very large amounts of water-insoluble tropomyosin. The quantity of actomyosin in the fiber is much smaller than in other kinds of muscle. Sarcosomes are few and small.

There are two features which may be connected with the well-developed tonic properties of these muscles, (a) tension persists long after active state has decayed to zero, and (b) during acetylcholine or direct current stimulation, resistance to stretch is very high and diminishes only gradually after stimulation has ended. These observations suggest the presence of two systems, one concerned with the active development of tension, the other with (passive) maintenance of that tension (Lowy and Millman, 1959b). A tetanic mechanism must be postulated for the maintenance of a steady level of tension, for in the absence of further activation passive tension decays to zero.

2. *Insect Flight Muscle*

The outstanding feature of the flight muscles in many insects is the speed and efficiency with which they oscillate, in some cases at a rate

linkages break at a very slow rate and are responsible for the phenomenon of passive tension. Further experiments (Lowy and Millman, unpublished results) now indicate that the linkages responsible for passive tension are of the same type as those formed during active state. Accordingly it appears that the kind of response (tonic or phasic) given by the ABRM depends on the rate at which linkages – all of one type – break, and this will be determined by the amount of 5-hydroxytryptamine-like substance present (see p. 305).

The structure of the ABRM has been investigated further by electron microscopy. The filaments are of two kinds, thin and thick, the latter showing paramyosin structure (see p. 297) (Hanson and Lowy, 1959 a, b; Philpott *et al.*, 1959). The two kinds of filaments are cross-linked by bridges (Hanson and Lowy, 1959 a, b) which resemble those found in striated muscles (Volume I, Chapter VII) and in the smooth muscles of *Loligo* and *Helix* (see p. 287) in their appearance and arrangement. The thin filaments are about 50 A in diameter; their length is not yet known. Using the ABRM, a comparison of transverse sections of extended and shortened muscles has shown that neither the diameters of the thick (paramyosin) filaments nor their lateral separation from each other changes on shortening (Hanson and Lowy, 1959 a, b). These results strongly suggest that the paramyosin filaments are discontinuous along the fiber, that they do not contract, and that they change their positions when the muscle shortens, i.e. they slide.

All these new structural results, together with evidence already discussed (see p. 284–5) indicate that "paramyosin smooth muscles" probably contract by a sliding filament mechanism. By analogy with striated muscles (Volume I, Chapter VII) it has been suggested (Hanson and Lowy, 1959 a, b) that in these smooth muscles too, the tension developed during active state is due to the formation of cross-linkages between thick and thin filaments. There is as yet no evidence that these linkages are actin myosin linkages, although actomyosin can be prepared from "paramyosin smooth muscles."

The problem of the function of filaments with paramyosin structure has been approached in a different way by Johnson *et al.* (1959) who studied glycerol-extracted preparations of the ABRM. When these were treated with ATP it was found that under certain conditions (pH 6.0, low ionic strength), isotonic shortening could be reversibly inhibited, whilst the capacity for "isometric tension development" remained unimpaired The conditions were the same as those which brought about precipitation ("crystallization") of paramyosin (water-insoluble tropomyosin) *in vitro*. Under conditions where paramyosin remained in solution *in vitro* (pH 6.7), glycerol-extracted preparations of the ABRM not only showed normal "isometric tension development" but also shortened readily. Johnson *et al.* interpreted these results as indicating that prolonged maintenance of tension in the ABRM is due to a "catch mechanism" mediated by the crystallization of paramyosin *in vivo*. This hypothesis was also supported by the finding (Johnson and Philpott, 1959) that the 145 A. axial periodicity characteristic of paramyosin was absent in electron micrographs of muscles which were fixed while relaxed However, Hanson and Lowy (1959 b) report that this fine structure is equally visible in the ABRM fixed either when relaxed or during a tonic response.

Lastly, it has been reported (Ruegg, 1958) that artificial fibers of water-insoluble tropomyosin prepared from the smooth adductor of *Pecten* can be plasticized by ATP, and that glycerol-extracted preparations (of the same muscle) in which actomyosin has been denatured by ethanol are similarly plasticized.

with each other along the length of the contractile element; where the arrays overlap, the two kinds of filaments are cross-linked. As a result of this construction, the structure is transversely banded, i.e. striated.

Striation of this kind appears to be associated with an ability to contract and relax rapidly—but it is not clear why. Perhaps such a construction allows the contractile element to be brought into action more quickly than would be possible if the filaments were more randomly arranged with respect to the intracellular stimulus conduction system. In other words, the presence of striation could be more significant for the mechanism by which the contractile element is stimulated than for a particular manner of contraction. Thus in some animals, striated muscles can be used for rather slow movements.

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Appendix

The tonic smooth muscles of lamellibranch molluscs

Since this chapter was written new information has been obtained about "paramyosin smooth muscles." To relate what is now known of their behavior and structure it will be useful first to mention again some of the observations (already referred to in the text) made in physiological experiments with the anterior byssal retractor muscle of *Mytilus* (ABRM) by Lowy and Millman (1959 a, b).

A specialized tonic muscle such as the ABRM can, by suitable periodic stimulation, be made to hold a high level of tension for several hours without fatigue. If stimulation is stopped, tension decays to zero, and the mechanism would therefore appear to be of a tetanic nature. However, the frequency at which the muscle has to be re-excited is very low compared with that required to maintain tension in a non-tonic smooth muscle like the mantle of squid (Prosser, 1950). This could be explained by the finding that in the ABRM, the decay of tension in a twitch can be two orders of magnitude slower than that of active state, and this also applies when active state is prolonged by stimulating the muscle for several seconds either with direct current or with acetylcholine. The tension which persists after active state has decayed to zero has been called passive tension.

Lowy and Millman (1959 b) put forward a hypothesis to account for these special properties of "paramyosin smooth muscles." They postulate that the tension developed during active state is due to the formation of linkages in a sliding filament actomyosin system, that linkages are also made which involve a second system (characterized by the presence of paramyosin structure), and that these latter

Casella, C. (1951). *Acta Physiol. Scand.* **21**, 380.
Chadwick, L. E. (1953). *In* "Insect Physiology" (K. D. Roeder, ed.), Wiley, New York.
Chapman, G. B. (1954). *J. Morphol.* **95**, 237.
Chapman, L. E., and Gilmour, D. (1940). *Physiol. Zool.* **13**, 398.
Cleland, K. W., and Slater, E. C. (1953). *Quart. J. Microscop. Sci.* **94**, 329.
Cohen, C. (1958). *J. Biophys. Biochem. Cytol.* **4**, 489.
Csapo, A. (1955). *In* "Modern Trends in Obstetrics and Gynaecology" (K. Bowes, ed.), Ser. II, Harper, New York.
Darwin, C. (1851). "A monograph of the Sub-Class Cirripedia. The Lepadidae." Ray Soc., London.
de Geer, C. (1776). Quoted by Chadwick, L. E. (1953).
Délage, Y., and Hérouard, E. (1897). "Traité de Zoologie Concrète. Tome V. Les Vermidiens." Schleicher, Paris.
Dorr, D., and Portzehl, H. (1954). *Z. Naturforsch.* 9b, 550.
Dubuisson, M., and Pezeu, M. H. (1947). *Compt. rend. soc. biol.* **141**, 800.
Dubuisson-Brouha, A. (1953). *Bull. classe sci. Acad. roy. Belg.* (5) **39**, 121.
Edwards, G. A. (1957). *Anat. Record* **128**, 542.
Edwards, G. A., Ruska, H., Santos, P. de S., and Vallejo-Friere, A. (1956). *J. Biophys. Biochem. Cytol.* **2**, *Suppl.* p. 143.
Edwards, G. A., Ruska, H., and de Harven, É. (1958). *J. Biophys. Biochem. Cytol.* **4**, 107.
Eggleton, M. G. (1934). *J. Physiol. (London)* **82**, 79.
Elliott, G. F., Hanson, J., and Lowy, J. (1957). *Nature* **180**, 1291.
Emery, C. (1887). *Mitt. Zool. Sta. Neapel* **7**, 371.
Engelmann, T. W. (1881). *Arch. ges. Physiol. Pfluger's* **25**, 538.
Ennor, A. H. and Rosenberg, H. (1952). *Biochem. J.* **51**, 606.
Esser, W. (1934). *Arch. biol. Paris* **45**, 377.
Fischer, E. (1947). *Ann. N. Y. Acad. Sci.* **47**, 783.
Fletcher, C. M. (1937). *J. Physiol. (London)* **90**, 233.
Fol, H. (1888). *Compt. rend. Acad. Sci. Paris* **106**, 306.
Gasser, H. S., and Hill, A. V. (1924). *Proc. Roy. Soc.* B **96**, 398.
Gilmour, D., and Calaby, J. H. (1953). *Enzymologia* **16**, 23.
Godeaux, J. (1954). *Bull. classe sci. Acad. roy. Belg.* (5) **40**, 948.
Goodall, M. C. (1958). *Yale J. Biol. and Med.* **30**, 224.
Greville, G. D., and Needham, D. M. (1955). *Biochim. et Biophys. Acta* **16**, 284
Grobben, C. (1881). *Arb. zool. Inst. Wien* **4**, 201.
Gruvel, A. (1896). *Compt. rend. Acad. Sci. Paris* **123**, 68.
Hall, C. E., Jakus, M. A., and Schmitt, F. O. (1945). *J. Appl. Phys.* **16**, 459.
Hamann, O. (1887). *Jena. Z. Naturw.* **21**, 87.
Hamann, O. (1889). *Jena. Z. Naturw.* **23**, 113.
Hanson, J. (1956a). *Biochim. et Biophys. Acta* **20**, 289.
Hanson, J. (1956b). *J. Biophys. et Biochem. Cytol.* **2**, 691.
Hanson, J. (1957). *J. Biophys. et Biochem. Cytol.* **3**, 111.
Hanson, J., and Huxley, H. E. (1955). *Symposia Soc. Exptl. Biol.* **9**, 228.
Hanson, J., and Lowy, J. (1957). *Nature* **180**, 906.
Hanson, J., and Lowy, J. (1959 a). *Nature, Lond.*, (in press).
Hanson, J., and Lowy, J. (1959 b). *J. Physiol. (London)* (in press).
Hanson, J., Lowy, J., Huxley, H. E., Bailey, K., Kay, C. M., and Ruegg, J.C. (1957). *Nature* **180**, 1134.
Haswell, W. (1889). *Quart. J. Microscop. Sci.* **30**, 31.
Hess, A., Cohen, A. I., and Robson, E. A. (1957). *Quart. J. Microscop. Sci.* **98**, 315.

References

Abbott, B. C. (1951). *J. Physiol. (London)* **112**, 438.
Abbott, B. C., and Aubert, X. (1952). *J. Physiol. (London)* **117**, 77.
Abbott, B. C., and Lowy, J. (1953). *J. Physiol. (London)* **120**, 50 P.
Abbott, B. C., and Lowy, J. (1955). *J. Physiol. (London)* **130**, 25 P.
Abbott, B. C., and Lowy, J. (1956a). *J. Marine Biol. Assoc. United Kingdom* **35**, 521
Abbott, B. C., and Lowy, J. (1956b). *Nature* **177**, 788.
Abbott, B. C., and Lowy, J. (1956c). *Nature* **178**, 147.
Abbott, B. C., and Lowy, J. (1957). *Proc. Roy. Soc.* **B 146**, 280.
Abbott, B. C., and Lowy, J. (1958a). *J. Physiol. (London)* **141**, 398.
Abbott, B. C., and Lowy, J. (1958b). *J. Physiol. (London)* **141**, 385.
Abbott, B. C., and Ritchie, J. M. (1951). *J. Physiol. (London)* **113**, 336.
Alexandrowicz, J. (1927). *Arch. zool. exptl. et gén.* **66**, 71.
Amberson, W. R., Smith, R. D., Chinn, B., Himmelfarb, S., and Metcalf, J. (1949). *Biol. Bull.* **97**, 231.
Apathy, S. (1893). *Z. wiss. Mikroskop.* **10**, 36, 319.
Apel, W. (1885). *Z. wiss. Zool.* **42**, 459.
Astbury, W. T. (1947). *Proc. Roy. Soc.* **B 134**, 303.
Bailey, K. (1942). *Biochem. J.* **36**, 121.
Bailey, K. (1948). *Biochem. J.* **43**, 271.
Bailey, K. (1956). *Pubbl. staz. zool. Napoli* **29**, 96.
Bailey, K. (1957). *Biochim. et Biophys. Acta* **24**, 612.
Baldwin, E., and Yudkin, J. (1949). *Proc. Roy. Soc.* **B 136**, 614.
Ballowitz, E. (1892). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **29**, 291.
Barnes, G. E. (1955). *J. Exptl. Biol.* **32**, 158.
Bear, R. S. (1944). *J. Am. Chem. Soc.* **66**, 2043.
Bear, R. S. (1945). *J. Am. Chem. Soc.* **67**, 1625.
Bear, R. S., and Cannon, C. (1951). *Nature* **168**, 684.
Bear, R. S., and Selby, C.C. (1956). *J. Biophys. Biochem. Cytol.* **2**, 55.
Bendall, J. R., and Davey, C. L. (1957). *Biochim. et Biophys. Acta* **26**, 93.
Biedermann, W. (1895). "Elektrophysiologie," p. 34. Fischer, Jena.
Boettiger, E. G. (1957a). *In* "Recent Advances in Invertebrate Physiology" (B. T. Scheer, ed.), p. 117. Univ. Oregon Publ.
Boettiger, E. G. (1957b). *In* "Physiological Triggers" (T. H. Bullock, ed.), p. 103. Am. Physiol. Soc., Washington, D. C.
Boettiger, E. G., and Furshpan, E. (1952). *Biol. Bull.* **102**, 200.
Boettiger, E. G., and Furshpan, E. (1954a). *Biol. Bull.* **107**, 305.
Boettiger, E. G., and Furshpan, E. (1954b). *J. Cellular Comp. Physiol.* **44**, 340.
Boettiger, E. G., and McCann, F. (1953). *Federation Proc.* **12**, 17.
Borg, F. (1926). *Zool. Bidr.* **10**, 181.
Bottazzi, P (1897). *J Physiol (London)* **21**, 1.
Bozler, E. (1928). *Z. vergleich. Physiol.* **8**, 372.
Bozler, E. (1930) *Z. vergleich. Physiol.* **12**, 579.
Brakenhoff, H. (1937). *Zool. Jahrb. Abt. Anat. u. Ontog. Tiere* **63**, 125.
Buchthal, F., and Weis-Fogh, T. (1956). *Acta Physiol. Scand.* **35**, 345.
Buchthal, F., Weis-Fogh, T., and Rosenfalck, P. (1957). *Acta Physiol. Scand.* **39**, 246.
Burfield, S. T. (1927). "*Sagitta*". Liverpool Marine Biological Committee Memoir, no. 28.
Caesar, R., Edwards, G. A., and Ruska, H (1957). *J. Biophys. Biochem. Cytol.* **3**, 867.
Caldwell, P. C. (1953). *Biochem J.* **55**, 458.

Casella, C. (1951). *Acta Physiol. Scand.* **21**, 380.
Chadwick, L. E. (1953). *In* "Insect Physiology" (K. D. Roeder, ed.), Wiley, New York.
Chapman, G. B. (1954). *J. Morphol.* **95**, 237.
Chapman, L. E., and Gilmour, D. (1940). *Physiol. Zoöl.* **13**, 398.
Cleland, K. [illegible] 29.
Cohen, C. (1 [illegible]
Csapo, A. (1 [illegible] (K. Bowes, ed.), Ser. II, Harper, New York.
Darwin, C. (1851). "A monograph of the Sub-Class Cirripedia. The Lepadidae." Ray Soc., London.
de Geer, C. (1776). Quoted by Chadwick, L. E. (1953).
Délage, Y., and Hérouard, E. (1897). "Traité de Zoologie Concrète. Tome V. Les Vermidiens." Schleicher, Paris.
Dorr, D., and Portzehl, H. (1954). *Z. Naturforsch.* 9b, 550.
Dubuisson, M., and Pezeu, M. H. (1947). *Compt. rend. soc. biol.* **141**, 800.
Dubuisson-Brouha, A. (1953). *Bull. classe sci. Acad. roy. Belg.* (5) **39**, 121.
Edwards, G. A. (1957). *Anat. Record* **128**, 542.
Edwards, G. A., Ruska, H., Santos, P. de S., and Vallejo-Friere, A. (1956). *J. Biophys. Biochem. Cytol.* **2**, *Suppl.* p. 143.
Edwards, G. A., Ruska, H., and de Harven, É. (1958). *J. Biophys. Biochem. Cytol.* **4**, 107.
Eggleton, M. G. (1934). *J. Physiol. (London)* **82**, 79.
Elliott, G. F., Hanson, J., and Lowy, J. (1957). *Nature* **180**, 1291.
Emery, C. (1887). *Mitt. Zool. Sta. Neapel* **7**, 371.
Engelmann, T. W. (1881). *Arch. ges. Physiol. Pflüger's* **25**, 538.
Ennor, A. H. and Rosenberg, H. (1952). *Biochem. J.* **51**, 606.
Esser, W. (1934). *Arch. biol. Paris* **45**, 377.
Fischer, E. (1947). *Ann. N. Y. Acad. Sci.* **47**, 783.
Fletcher, C. M. (1937). *J. Physiol. (London)* **90**, 233.
Fol, H. (1888). *Compt. rend. Acad. Sci. Paris* **106**, 306.
Gasser, H. S., and Hill, A. V. (1924). *Proc. Roy. Soc.* B **96**, 398.
Gilmour, D., and Calaby, J. H. (1953). *Enzymologia* **16**, 23.
Godeaux, J. (1954). *Bull. classe sci. Acad roy. Belg.* (5) **40**, 948.
Goodall, M. C. (1958). *Yale J. Biol. and Med.* **30**, 224.
Greville, G. D., and Needham, D. M. (1955). *Biochim. et Biophys. Acta* **16**, 284
Grobben, C. (1881). *Arb. zool. Inst. Wien* **4**, 201.
Gruvel, A. (1896). *Compt. rend. Acad. Sci. Paris* **123**, 68.
Hall, C. E., Jakus, M. A., and Schmitt, F. O. (1945). *J. Appl. Phys.* **16**, 459.
Hamann, O. (1887). *Jena. Z. Naturw.* **21**, 87.
Hamann, O. (1889). *Jena. Z. Naturw.* **23**, 113.
Hanson, J. (1956a). *Biochim. et Biophys. Acta* **20**, 289.
Hanson, J. (1956b). *J. Biophys. et Biochem. Cytol.* **2**, 691.
Hanson, J. (1957). *J. Biophys. et Biochem. Cytol.* **3**, 111.
Hanson, J., and Huxley, H. E. (1955). *Symposia Soc. Exptl. Biol.* **9**, 228.
Hanson, J., and Lowy, J. (1957). *Nature* **180**, 906.
Hanson, J., and Lowy, J. (1959 a). *Nature, Lond.*, (in press).
Hanson, J., and Lowy, J. (1959 b). *J. Physiol. (London)* (in press).
Hanson, J., Lowy, J., Huxley, H. E., Bailey, K., Kay, C. M., and Ruegg, J.C. (1957). *Nature* **180**, 1134.
Haswell, W. (1889). *Quart. J. Microscop. Sci.* **30**, 31.
Hess, A., Cohen, A. I., and Robson, E. A. (1957). *Quart. J. Microscop. Sci.* **98**, 315.

Hibbs, R. G. (1956). *Am. J. Anat.* **99**, 17.
Hill, A. V. (1926). *Proc. Roy. Soc.* **B100**, 108.
Hill, A. V. (1938). *Proc. Roy. Soc.* **B126**, 136.
Hill, A. V. (1949). *Proc. Roy. Soc.* **B136**, 399.
Hill, A. V. (1950). *Proc. Roy. Soc.* **B137**, 273.
Hill, A. V. (1952). *Proc. Roy. Soc.* **B139**, 464.
Hill, A. V. (1953). *Proc. Roy. Soc.* **B141**, 161.
Hill, D. K. (1949). *J. Physiol (London)* **108**, 292.
Hobson, G. E., and Rees, K. R. (1955). *Biochem. J.* **61**, 549.
Hodge, A. J. (1952). *Proc. Natl. Acad. Sci. U. S. 5.* **38**, 850.
Hodge, A. J. (1955). *J. Biophys. Biochem. Cytol.* **1**, 361.
Hodge, A. J., Huxley, H. E., and Spiro, D. (1954). *J. Exptl. Med.* **99**, 201.
Horridge, A. (1954). *Quart. J. Microscop. Sci.* **95**, 85.
Hoyle, G. (1957). "Comparative Physiology of the Nervous Control of Muscular Contraction." Cambridge Univ. Press, London and New York.
Hoyle, G., and Lowy, J. (1956). *J. Exptl. Biol.* **33**, 295.
Humphrey, G. (1949a). *Physiol. Comparata et Oecol.* **1**, 89.
Humphrey, G. (1949b). *Physiol. Comparata et Oecol.* **1**, 366.
Huxley, A. F. (1957). *Progr. in Biophys. and Biophys. Chem.* **7**, 255.
Huxley, H. E. (1951). *Discussions Faraday Soc.* **11**, 148.
Huxley, H. E. (1953). *Proc. Roy. Soc.* **B141**, 59.
Huxley, H. E. (1959). *In* "The Cell" (J. Brachet and A. Mirsky, eds.). Academic Press, New York, *in press.*
Huxley, H. E., and Hanson, J. (1957). *Abstr. 1st European Electron Microscopy Congr. Stockholm* p. 202.
Huxley, H. E., and Kendrew, J. C. (1952). *Nature* **170**, 882.
Jakus, M. A., Hall, C. E., and Schmitt, F. O. (1944). *J. Am. Chem. Soc.* **66**, 313.
Jen, M. -H. and Tsao, T. -C. (1957). *Sci. Sinica (Peking)* **6**, 317.
Jensen, M. (1956). *Phil. Trans. Roy. Soc. London Ser.* **B239**, 511.
Jewell, B., and Wilkie, D. R. (1958). *J. Physiol. (London)* **143**, 515.
Johnson, W. H., Kahn, J., and Szent-Gyorgyi, A. G. (1959). *Science* (in press).
Johnson, W. H. and Philpott, D. E. (1959). Abstracts of papers presented at American Biophysical Society meeting. Pittsburg, 1959.
Jordan, H. J. (1938). *Ergeb. Physiol. u. exptl. Pharmakol.* 40, 437.
Katz, B. (1939). *J. Physiol.* **96**, 45.
Kay, C. (1958). *Biochim. et Biophys. Acta* **27**, 469.
Keilin, D (1925). *Proc. Roy. Soc.* **B98**, 312.
Kiernick, E (1905). *Zool. Anz.* **29**, 610.
Koehler, R. (1889). *Arch biol. Paris* **9**, 311.
Kominz, D. R , Saad, F., and Laki, K. (1957a). *Nature* **179**, 206.
Kominz, D. R., Saad, F., Gladner, J. A , and Laki, K. (1957b). *Arch. Biochem.* **70**, 16.
Kominz, D. R., Saad, F., and Laki, K. (1958). Proceedings of Conference on the Chemistry of Muscular Contraction. Tokyo, 1957, p. 66.
Krasińska, S. (1914). *Z. wiss. Zool.* **145**, 169.
Krijgsman, B. J. (1956). *Biol Revs. Cambridge Phil. Soc.* **31**, 288.
Krijgsman, B J., and Divaris, G A. (1955). *Biol. Revs. Cambridge Phil. Soc.* **30**, 1.
Krogh, A., and Weis-Fogh, T. (1951). *J. Exptl. Biol.* **28**, 344.
Kukenthal, W., and Krumbach, T. (1933). "Handbuch der Zoologie," Vol. 5, pt. 2. De Gruyter, Berlin.
Kupelweiser, H. (1906). *Zoologica* **19**, Pt. 47, 1.
Levenbook, L., and Williams, C. M. (1956). *J Gen. Physiol.* **39**, 497.
Lewis, S. E., and Slater, E. C (1954) *Biochem. J.* **58**, 207.

Leydig, F. (1859). *Arch. Anat. Physiol. Lpz.* 1859, 149.
Locker, R. H., and Schmitt, F. O. (1957). *J. Biophys. Biochem. Cytol.* **3**, 889.
Lorand, L., and Moos, C. (1956). *Nature* **117**, 1239.
Lotmar, W., and Picken, L. E. R. (1942). *Helv. Chim. Acta* **25**, 538.
Lowy, J. (1953). *J. Physiol. (London)* **120**, 129.
Lowy, J., and Millman, B. M. (1959a). *J. Physiol.* (*London*) **146**, 32P.
Lowy, J., and Millman, B. M. (1959b). *Nature*, **183**, 1730.
Lowy, J., and Millman, B. M. (1959a). *J. Physiol.*, 146, 32 P. (1959 b). *Nature, Lond.*, (in press).
Marceau, F. (1905a). *Trav. Lab. Stat. Biol., Arcachon*, 8th. year p. 48.
Marceau, F. (1905b). *Arch. anat. microscop.* **7**, 495.
Marceau, F. (1909). *Arch. zool. exptl. gén.* series 5, volume 2, 295.
Maréchal, G., and Aubert, X. (1958). *Arch. intern. physiol. et biochem.* **66**, 91.
Margo, T. (1860). *Sitzber. Akad. Wiss. Wien Math. naturw. Kl.* **39**, 559.
Maruyama, K. (1954a). *Enzymologia* **17**, 90.
Maruyama, K. (1954b). *Biochim. et Biophys. Acta* **14**, 284.
Maruyama, K. (1955). *Biochim. et Biophys. Acta* **16**, 589.
Maruyama, K. (1957). *Sci. Papers Coll. Gen. Educ. Univ. Tokyo* **7**, 213.
Maruyama, K. (1958a). *Enzymologia* **19**, 22.
Maruyama, K. (1958b). *Enzymologia* **19**, 28.
Maruyama, K. (1958c). *Biol. Bull.* **114**, 95.
Maruyama, K., and Matsumiya, H. (1957). *J. Biochem. Tokyo* **44**, 537.
Maruyama, K., and Tonomura, Y. (1957). *J. Research Inst. Catalysis Hokkaido* **5**, 55.
Matsumiya, H., Monita, F., Kitagawi, S., Yagi, K., and Tonomura, Y. (1957). *J. Biochem. Tokyo* **44**, 347.
Menkes, J., and Csapo, A. (1952). *Endocrinology* **50**, 37.
Merton, H. (1911). *Zool. Anz.* **37**, 561.
Mettenheimer, C. (1860). *Arch. Anat. Physiol., Lpz.* 1860, 361.
Mihályi, E., and Szent-Gyorgyi, A. G. (1953). *J. Biol. Chem.* **201**, 211.
Mommaerts, W. F. H. M., and Green, I. G. (1954). *J. Biol. Chem.* **208**, 833.
Motley, H. L. (1933). *J. Morphol.* **54**, 415.
Mueller, J. F. (1929). *Z. Zellforsch. u. mikroskop. Anat.* **8**, 361.
Needham, D. M., and Cawkwell, J. M. (1956). *Biochem. J.* **63**, 337.
Needham, D. M., and Cawkwell, J. M. (1958). *Biochem. J.* **68**, 31 P.
Nicol, J. (1951). *J. Exptl. Biol.* **28**, 22.
Perry, S. V., and Corsi, A. (1958). *Biochem. J.* **68**, 5.
Philpott, D. E., Kahlbrock, M., and Szent-Gyorgyi, A. G. (1959). *J. Ultrastructure Research* (in press)
Plate [illegible] *...t. Anat. u. Ontog. Tiere* **3**, 487.
..., 2.
...5, 249.
...t. Forsch. **4**, 163.
... Forsch. **33**, 605.
...**8**, 144.
... et gén. **69**, 1.
Pringle, J. W. S. (1949). *J. Physiol. (London)* **108**, 226.
Pringle, J. W. S. (1954). *J. Physiol. (London)* **124**, 269.
Pringle, J. W. S. (1957). "Insect Flight." Cambridge Univ. Press, London and New York.
Prosser, C. L. (1950). *In* "Comparative Animal Physiology" (C. L. Prosser, ed.), p. 576. Saunders, Philadelphia.

Prosser, C. L., (1950). *In* "Comparative Animal Phyciology" (C. L. Prosser, ed.), p. 593. Saunders, Philadelphia.
Rauther, M. (1914). *Zool. Anz.* **43**, 561.
Reichensperger, A. (1912). *Z. wiss. Zool.* **101**, 1.
Remane, A. (1933). *In* "Handbuch der Zoologie" (W. Kukenthal and T. Krumbach, eds.), Vol. 2, pt. 1, Heft 4, p. 121. De Gruyter, Berlin.
Retterer, E., and Lelièvre, A. (1909). *Compt. rend. soc. biol.* **66**, 571.
Rey, C. (1956). *Biochim. et Biophys. Acta* **19**, 300.
Ritchie, A. D. (1928). "The Comparative Physiology of Muscular Tissue," p. 111. Cambridge Univ. Press, London and New York.
Ritchie, J. M. (1954a). *J. Physiol. (London)* **123**, 633.
Ritchie, J. M. (1954b). *J. Physiol. (London)* **124**, 605.
Ritchie, J. M. (1954c). *J. Physiol.* **126**, 155.
Ritchie, J. M., and Wilkie, D. R. (1955). *J. Physiol.* **130**, 488.
Roeder, D. K. (1951). *Biol. Bull.* **100**, 95.
Roeder, D. K. (1953). *In* "Insect Physiology" (D. K. Roeder, ed.), Wiley, New York.
Romanes, G. J. (1885). "Jelly-fish, Star-fish and Sea-urchins." Kegan Paul, London.
Roskin, G. (1925a). *Acta Zool. Stockholm.* **6**, 253.
Roskin, G. (1925b). *Z. Zellforsch. u. mikroskop. Anat.* **2**, 766.
Ruegg, J. C. (1957). *Helv. Physiol. et Pharmacol. Acta* **15**, C33.
Ruegg, J. C., (1958). *Biochem., J.*, 69, 46 P.
Sacktor, B. (1955). *J. Biophys. Biochem. Cytol.* **1**, 1.
Sarkar, N. K. (1951). *Enzymologia* **14**, 288.
Schlote, F. -W. (1957). *Z. Naturforsch.* **12b**, 647.
Schmidt, W. J. (1936). *Z. Zellforsch. u. mikroskop. Anat.* **24**, 525.
Schmidt, W. J. (1938). "Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma." Borntraeger, Berlin.
Schmitt, F. O., Bear, R. S., Hall, C. E., and Jakus, M. A. (1947). *Ann. N. Y. Acad. Sci.* **47**, 799.
Schneider, K. C. (1902). "Lehrbuch der vergleichenden Histologie der Tiere." Fischer, Jena.
Schwalbe, G. (1869). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **5**, 205.
Selby, C. C., and Bear, R. S. (1956). *J. Biophys. Biochem. Cytol.* **2**, 71.
Sheng, P.-K., and Tsao, T.-C. (1954). *Sci. Sinica (Peking)* **4**, 157.
Sotavalta, O. (1947). *Acta entomol. Fennica* **4**, 1.
Thaoi, N. V, Roche, J., Robin, Y., and Thiem, N. V. (1953). *Compt. rend. soc. biol.* **147**, 1241.
Tiegs, O. (1955). *Phil. Trans. Roy. Soc. London* **B238**, 221.
Tonomura, Y. (1956). *J. Research Inst. Catalysis Hokkaido* **4**, 87.
Tonomura, Y, Yagi, K., and Matsumiya, H. (1955). *Arch. Biochem.* **59**, 76.
Tonomura, Y., Yagi, K., and Matsumiya, H. (1956). *Arch. Biochem.* **64**, 466.
Tsao, T.-C, Tan, P.-H, and Peng, C.-M. (1955). *Sci. Sinica (Peking)* **5**, 91.
Twarog, B. M. (1954). *J. Cellular Comp. Physiol* **44**, 141.
Ulbrecht, G., and Ulbrecht, M. (1952). *Z Naturforsch.* **7b**, 434.
Ulbrecht, G, Ulbrecht, M, and Weber, A. -M. (1954). *Biochim. et Biophys. Acta* **13**, 564.
Villafranca, G. W. de (1955) *Biol. Bull.* **108**, 113.
Watanabe, M. I., and Williams, C M. (1951). *J. Gen. Physiol.* **34**, 675.
Weber, H. H. (1956) *Fortschritte der Zoologie* **10**, 304.
Weis-Fogh, T. (1952). *Phil. Trans Roy. Soc London* **B237**, 1.
Weis-Fogh, T. (1956a). *J. Exptl Biol.* **33**, 668.

Weis-Fogh, T. (1956b). *Phil. Trans. Roy. Soc. London* **B239**, 459.
Wesenberg-Lund, C. (1939). "Biologie der Süsswassertiere." Springer, Vienna.
Wigglesworth, V. B. (1950). "The Principles of Insect Physiology." Methuen, London.
Wigglesworth, V. B. (1956). *Quart. J. Microscop. Sci.* **97**, 89.
Wilkie, D. R. (1954). *Progr. in Biophys. and Biophys. Chem.* **4**, 288.
Wilkie, D. R. (1956a). *Brit. Med. Bull.* **12**, 177.
Wilkie, D. R. (1956b). *J. Physiol. (London)* **131**, 527.
Worthington, C. R. (1956). *Biochim. et Biophys. Acta* **19**, 176.
Yagi, K. (1957). *J. Biochem. Tokyo* **44**, 337.
Yonge, C. M. (1928). *Phil. Trans. Roy. Soc. London* **B216**, 221.
Yoshimura, K. (1955). *Mem. Fac. Fisheries Hokkaido* **3**, 159.
Zelinka, K. (1928). "Monographie der Echinodera." Engelmann, Leipzig.

CHAPTER X

Motor End-Plate Structure

R. Couteaux

I. INTRODUCTION

Over a long period during which silver impregnations were almost exclusively used in investigations on the fine structure of motor end-plates, the preparations, at times impressive, obtained either with "reduced silver nitrate methods," or with "ammoniacal silver methods" have often resulted only in fruitless arguments.

While the silver impregnations have been, and continue to be, of great value for an over-all survey of muscular innervation, as well as for a general view of nerve terminal arborization, they have proved inadequate for a cytological study of the end-plate. Although they have in skilled hands produced striking pictures, there are serious drawbacks to many of these methods. For example, a mediocre, and even sometimes really bad, fixation often has to be used; or they may stain deeply filamentous structures other than the neurofibrils. This lack of specificity, which has not always been sufficiently considered, may give rise to certain misinterpretations.

Another serious shortcoming of the silver methods used in studying motor end-plates is their inability to show clearly the cytoplasmic boundaries. No reliable information can be obtained concerning the nature of the connection joining the motor nerve fiber and muscle fiber

when using techniques which leave the surface membranes practically unstained.

Rapid progress in our knowledge of the structure of motor end-plates has been made with methods other than silver impregnations; these methods allow staining of surface membranes or at least one of their components. By means of postvital stainings it has been possible to *delimit the sarcoplasm at the neuromuscular junction. Localization* with histochemical techniques of cholinesterase activity, located at the level of the sarcoplasm surface membrane, has facilitated observation of this membrane, and confirmed conclusions drawn from the results of postvital stainings. In addition, the use of the electron microscope has filled up numerous gaps which previously existed. This new technique, allowing the best fixations now available to cytologists, enables observations to be made with a resolution about one hundred times higher than before, and offers motor end-plate images on which plasma membranes are clearly seen.

Although the application of these methods is of recent date, they have already settled some long-standing debates raised by the study of the vertebrate motor end-plates.

It has been demonstrated that the plasma membranes, which limit respectively the nerve fiber and the striated muscle fiber, are present at their junction, as well as at all other points of their surface, and that they offer everywhere the same general structural patterns. Thus, the existence of a structural discontinuity between the nerve and muscle fibers is firmly established, confirming an important point of the neuron theory.

The electron micrographs have, on the other hand, shown that at the motor end-plate the surface membranes of the axoplasm and sarcoplasm are in immediate contact. Neither a collagenous membrane nor an endothelial membrane of the type forming the lamellar sheath of the nerve is found between the nerve ending and the sarcoplasm, and even the teloglia associated with each of the nerve ending branches leave the synaptic side of the branch exposed. In grown animals, the motor end-plates are the only points of the muscle at which the axoplasm of the motor nerve fiber and the sarcoplasm are in such proximity to each other. At all other points, a more or less thick layer of collagenous fibrils and sheaths surrounding the axon are interposed between the axoplasm and the sarcoplasm.

If the motor neuromuscular junction consisted only in the apposition

of two plasma membranes, its morphological study would be reduced to specifying the site of the junction, and the form and dimensions of the apposition surface of the pre- and postsynaptic membranes.

In fact, the motor nerve fiber and the muscle fiber each show, in the immediate vicinity of their junction, several distinguishing characteristics which they exhibit nowhere else, and which confer upon the motor junctional region a structural originality in its pre- and postsynaptic components.

Following the development of the striated muscle, one may observe the motor nerve ending and junctional sarcoplasm progressively acquiring their distinguishing characteristics, some of which probably result from local interactions between the axoplasm and sarcoplasm, interpretable as late inductions of the organogenesis. In regard to the sarcoplasm, the morphological features of the subneural area, and the appearance at its level of a high acetylcholinesterase activity are typical signs of this local, and obviously epigenetic, differentiation. Further study of motor end-plates, more particularly by new cytochemical techniques, will doubtless soon reveal other signs of specialization of the junctional sarcoplasm.

In this paper, the structure of the motor end-plate will be considered from a very general point of view, without discussing in detail the particularities which it exhibits in each type of striated muscle and in the different zoological divisions. Information concerning the comparative morphology of motor end-plates may be found in several reviews (Hines, 1927; Hinsey, 1934; Tiegs, 1953) for all classes of vertebrates, and in a very recent monograph in which Hoyle (1957) deals with "nervous control of the muscular contraction" in both vertebrates and invertebrates.

Regarding the pathological changes which may affect the structure of the motor end-plates, a paper by Coërs (1955) sets forth the principal known data.

II. EARLY OBSERVATIONS

Before the year 1840, histologists agreed with Valentin (1836) and Emmert (1836) that nerves possessed bow-shaped endings in striated muscles, the ultimate nerve branches joining up and continuing in one another. This opinion denied, in effect, the existence of true nerve endings with direct connexions between the motor nerve and each muscle fiber.

From Doyère (1840), we have our first notion of a nerve ending which ensures the close union of nerve and muscle fiber. But his conclusion dealt with the motor endings of an invertebrate, *Milnesium tardigradum*, endowed as all tardigrades with smooth muscle fibers only; the importance of this finding, confirmed by Quatrefages (1843) on the muscles of a gasteropode mollusc, *Eolidina paradoxa*, was not immediately appreciated by those histologists studying striated muscles in vertebrates.

It was not until 1847 that Wagner's observations on the striated muscles of the frog shed doubt for the first time on the existence of the terminal bows in striated muscles of vertebrates, described by Valentin and Emmert.

Wagner was the first to reveal two fundamental features of the neuromuscular junction of vertebrates; he concluded from his studies of the hyoidean muscles of the frog that the motor nerve fiber, after branching, loses its myelin sheath and closely connects with the muscle fiber.

Two other important characteristics, although less constant throughout the various types of neuromuscular junctions found in striated muscles, were subsequently discovered. The first was described by Kuhne (1862), w [illegible] ing the sarcolemma, [illegible] a terminal arborization; the second was described by Rouget (1862), who reported the presence, in the muscles of reptiles, birds, and mammals, of a flattened heap of granular nucleated substance at the level of the neuromuscular junction, which he interpreted as the spreading of the axis cylinder substance at the surface of the myofibrils and which he called the "end-plate" ("plaque terminale").

At the time of his discovery of the end-plate, Rouget did not perceive the nerve ending itself. It was Krause (1863) who, the following year, described the terminal branching at the level of the end-plate on the retractor muscle of a cat's eye, which he called "motor end-plate" ("motorische Endplatte"). This term is often applied today, conveniently but improperly, to all neuromuscular junctions, whether or not they present a plate of granular nucleated protoplasm.

The first really detailed observations on the structure of the motor end-plate were made by Ranvier (1878) and Kuhne (1883, 1887, 1892). All the later morphological research is based on the work of these two authors, especially on the very impressive paper by Kuhne, which appeared in 1887.

III. GENERAL VIEW OF THE MOTOR END-PLATE

For a general description of the vertebrate motor end-plate, we shall refer mainly to end-plates of reptiles and mammals.

The accumulation of nuclei and granular cytoplasm of the junctional area forms a rounded heap at the surface of the myofibrillar bundle. It is this plate-like heap of protoplasm lying against the myofibrillar bundle and at the level of the ramification of the nerve ending (Fig. 1) that Kühne called the sole.



FIG. 1. Drawing of two motor end-plates of guinea pig (m. gastrocnemius internus). Silver preparation.

Some of the various nuclei observed in the vicinity of the nerve ending are closely connected with the terminal nerve branches. These are the nuclei described by Ranvier (1878) as "arborization nuclei." Then there are the "fundamental nuclei" of Ranvier (Kuhne's "sole nuclei", 1864), which are larger and much less stainable than the former, and which contain much larger nucleoli. In addition to these two categories of nuclei, there are the endothelial nuclei of the blood capillaries, sometimes very closely attached to the end-plate, and lastly, the "vaginal nuclei" of Ranvier, which are not always present, and which are linked to the endothelial and collagenous coating prolonging Henle's sheath of the nerve fiber over the end-plate.

Of these four kinds of nuclei, observable at junction level, the only ones which are frequently easy to identify are the fundamental nuclei;

these are generally distinguished by their size and especially their structure. But this is not the case for the arborization nuclei, the endothelial nuclei of the blood capillaries, and the vaginal nuclei, at least in adult muscles. This doubtless explains the long, inconclusive debate between Kuhne and Ranvier on the end-plate nuclei, and the numerous controversies on this subject which have arisen constantly since the end of the last century.

Data obtained from a study of the development of the end-plate and from an analysis of its structure in the adult, both with light and electron microscopes, enable us to give a coherent interpretation of the highly complex neuromuscular junction, summarized by the schema of Fig. 2.

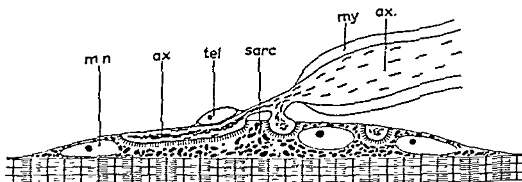


Fig. 2. Schematic drawing of a motor end-plate seen in a longitudinal section of the muscle fiber. ax., axoplasm with its mitochondria; my., myelin sheath; tel., teloglia; sarc., sarcoplasm with its mitochondria; m.n., muscle nuclei (fundamental nuclei). The terminal nerve branches lie in "synaptic gutters" or "troughs." Immediately under the interface axoplasm-sarcoplasm, the ribbon-shaped subneural lamellae, cut transversely, may be seen as rodlets. The bell-mouth by which the Henle's sheath ends above the motor end-plate has not been represented.

After the loss of its myelin sheath, the motor axon branches and connects with the sarcoplasm. The nerve branches are accompanied by teloglia (terminal Schwann cells), to which the arborization nuclei belong; the branches occupy hollows of widely varying depth, shape, and size according to the animal species considered. These "synaptic gutters" or "synaptic troughs" are hollowed out into the sole, a flattened-out heap of sarcoplasm rich in nuclei and mitochondria.

In spite of its close connections with each terminal nerve twig, teloglia does not penetrate the interior of the synaptic gutters, and does not come between the axoplasm and sarcoplasm.

By means of selective stainings, it is possible with a light microscope

to distinguish the "interface," at the level of which axoplasm and sarcoplasm are joined. On a section cut through a motor end-plate, this interface appears as a thin line, to which are attached by one of their edges on the sarcoplasm side the elongated ribbon-shaped lamellae of the subneural apparatus. When they are cut transversally and observed with a light microscope, these subneural lamellae appear as rodlets measuring generally about one micron in length. They are always perpendicular to the axoplasm and sarcoplasm apposition surface. The juxtaposition of these rodlets, situated on the deeper face of the nerve terminals, results in a picture resembling that of a palisade or picket fence.

On a front view of a motor end-plate, the lamellae, which run under the nerve terminals, appear as a fingerprint. Their orientation differs widely according to their position in the subneural apparatus; they are of unequal lengths and generally more close-set near the edges of gutters.

After this brief description of the main morphological features of the motor-end plate, we shall proceed to a more detailed analysis of its diverse components.

IV. TERMINAL AXOPLASM

As a result of extensive work, Kuhne (1887), using a gold method, Dogiel (1890), and Retzius (1892), with methylene blue, had already described the general form of motor nerve endings in each class of vertebrates. Their description was supported by numerous and excellent plates.

Subsequently, many other investigators using the same methods, as well as silver impregnations, confirmed and completed previous observations; but, although these methods permit a general survey of various types of motor nerve endings, they unfortunately provide very little reliable information concerning their fine structure. They are indeed quite often used, either in fresh tissues, or after poor fixations, and in both cases it is difficult to know which artifacts are included in the stained structures.

These staining methods leave, moreover, the border line separating the terminal axoplasm from the teloglia practically unstained.

In addition, because of their small size, most of the axoplasm components cannot be clearly seen, if at all, with the light microscope.

The nerve ending, as described by Kuhne, is made up of two parts, differentially stained by his gold method. The first part, stained deep

violet, is axial; this is the "axialbaum." The second part, the "stroma," stained red, encloses the first.

When the structure described by Kuhne as the stroma is located at the level of the still myelinated part of the axon preceding the branching, it must be admitted that this structure, found inside the myelin sheath, is part of the axoplasm.

The nature of what he designated under the name of stroma at the level of the nerve ending itself is much more uncertain. As we shall see in connection with the teloglia, it seems that Kuhne's stroma brings together quite different structures.

Kuhne observed that the stroma is not quite a homogeneous structure, being more granular in its peripheral part. Contrary to a rather widely held opinion, he never referred to the stroma as a striated layer. There is an obvious confusion here with a superficial fringe ("Borstensaum"), also described by Kuhne (1883), the significance of which will be discussed later. According to Kuhne, this fringe belongs to the membrane coating the end-plate. In his paper of 1887 (p. 21), and, in particular, in the legends accompanying the drawings of the fringe, he invariably and carefully distinguished it from the stroma.

Most of the investigators who have stained the nerve arborization of motor end-plates with methylene blue, by intravascular injection of the staining solution, or who have placed the excised muscle tissue in direct contact with the staining solution, have often observed much more deeply stained filament in the axis of the arborization branches (Dogiel, 1890; Feist, 1890; Kulchitsky, 1924; Tiegs, 1932; Tello, 1944; Couteaux, 1947).

It may happen that the axial filament is the only stained part of the nerve ending, but this picture is rarely obtained; in general, the axial filament stands out from the rest of the arborization which is pale blue by its deep blue color. A quite similar picture may sometimes be observed at the level of the axon myelinated part; a deep blue filament, occupying the axis of the nerve fiber, is separated from the myelin sheath by a poorly stained or unstained space.

Of all the methods used to selectively stain the motor nerve ending, those using silver gave the most clear-cut pictures, especially in the hands of Cajal (1904, 1925), Tello (1905, 1944), Boeke (1909, 1911, 1927), Lawrentjew (1928)

Silver impregnation discloses a neurofibrillar framework in the nerve arborization, prolonging the neurofibrillar bundle of the nerve fiber at

the level of the branches of this ending (Figs. 1 and 3; Plate I, a–c, e–g).

Many investigators who have studied the neurofibrillar framework of nerve arborization have pointed out that the neurofibrillar bundles of the terminal branches often end in an olive-shaped enlargement, a small racket-like net, in loops, or in rings.

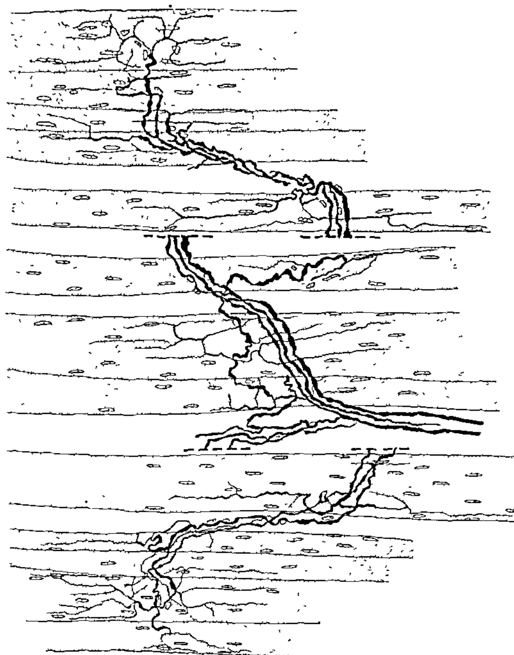


FIG. 3 Drawing of frog terminal arborizations (abdominal muscle). Silver preparation. The three median muscle fibers have been displaced in relation to the other fibers in order to show entirely their motor innervation. Magnification: × 190.

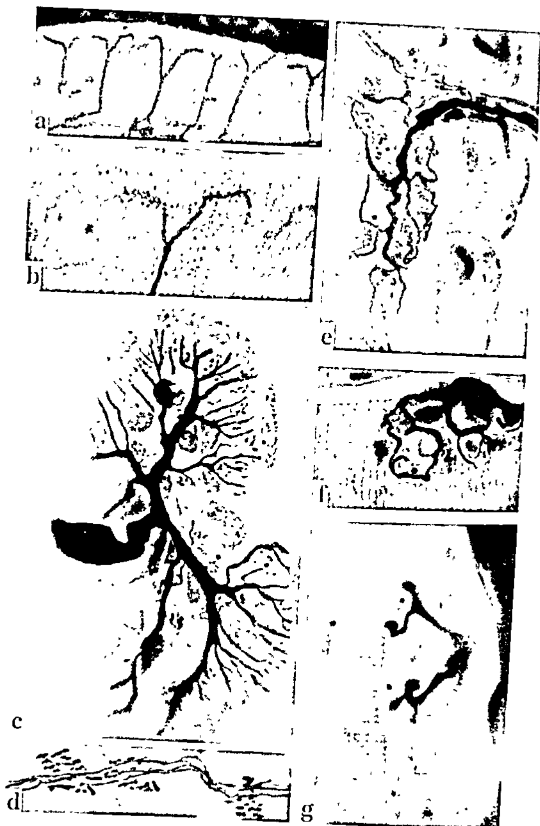


PLATE I

The neurofibrillar bundle, often very slender, which is found inside each of the nerve terminal branches, is surrounded by a clear space similar to the one observed, following staining by the methylene blue method, around a deeply stained axial filament.

It is quite probable that this clear space is, to a certain degree at least, the result of a fixation artifact, resulting from either a shrinkage of the entire axoplasm or the condensation of a fibrillar component. It is also likely that the teloglia, whose relationship with the nerve branches will be described later, is part of the perifibrillar layer.

The assumption put forward by several authors, principally Cajal (1925), to the effect that a peripheral layer of axoplasm lacking the fibrillar component always exists at the level of the terminal nerve branches cannot be entirely rejected today; the presence of such a layer would also explain the presence of a clear space surrounding the neurofibrillar bundle.

The only inclusions of the terminal axoplasm observable with the light microscope are very small granules, which can be stained either by Regaud's technique or by Altmann's aniline fuchsin with fixation by osmic mixtures. From their special stainability, it may be concluded that, in all probability, these granules are mitochondria. They appear more numerous at the level of the nerve ending than in the other regions of the motor axon.

When examined with the electron microscope (Fig. 4), these tiny granules, observable with the light microscope inside the nerve branches, exhibit the typical internal structure of mitochondria (Robertson, 1956).

Electron microscopy has further shown that, in addition to the

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PLATE I. Neuromuscular junctions of animal species belonging to different zoological divisions. (a) and (b): Motor nerve endings at the surface of an insect muscle fiber (*Musca domestica*, thoracic muscle). Silver method. Magnification: (a) × 520; (b) × 1,600 (original photomicrographs from Auber), (c) Motor end-plate of a Selachian muscle fiber (*Raja clavata*, pelvic fin muscle). Silver method. Magnification: × 800 (original photomicrograph from Barets). (d): Motor nerve endings at the surface of "slow" muscle fibers of the frog (m. rectus abdominis) Methylene blue method. Magnification: × 300 (original photomicrograph from Barets). (e)· Motor nerve ending ("twitch system") of the frog (m. rectus abdominis). The neuromuscular junction represented here, more concentrated than usually is the case in the frog muscle, effects the transition between the end-bush (see Fig. 3) and the true end-plate. Magnification: × 480 (from Couteaux, 1947). (f): Motor end-plate of the guinea pig (m. gastrocnemius internus). Silver method. Magnification· × 750. (g): Motor end-plate of man (larynx muscle). Silver method. Magnification. × 750 (original photomicrograph from Barets). *As frequently seen in human muscles, the nerve terminal branches are connected with the muscle fibers only by button-shaped extremities.*

mitochondria, the terminal axoplasm contains some inclusions which are much more numerous and much smaller (300–500 A.). These "vesicles" have been described in the motor nerve endings of the striated muscle by Palade and Palay (1954) and by Robertson (1956); they appear to be homologous to the presynaptic "vesicles" described for some interneuronal synapses.

In spite of these recent findings, there still exist some important gaps

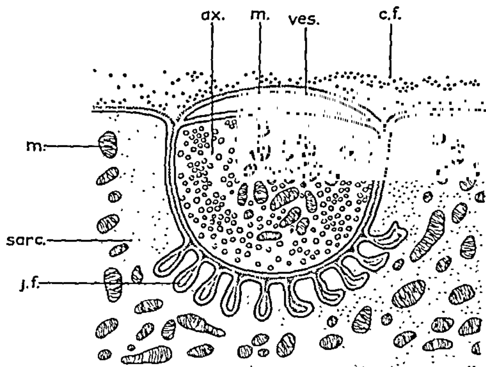


Fig. 4. Schematic drawing of a "synaptic gutter" or "trough," seen in cross section. ax., axoplasm; m., mitochondria; ves., vesicles; sarc., sarcoplasm; j.f., junctional folds; c.f., collagen fibrils (after Robertson, 1956, slightly modified).

in our knowledge of the fine structure of the terminal axoplasm. Studies made with the electron microscope have not yet allowed a satisfactory interpretation of the different pictures obtained with gold, silver, and methylene blue methods. In particular, neurofibrils of a nerve ending stained in the usual manner continue to pose a difficult problem. It seems probable today that the neurofibrils are a result of the artificial agglutination of submicroscopic filaments, called "neurofilaments"; observations made on nerve fibers of the leech (Couteaux, 1957a) suggest that this agglutination depends above all on the manner the fixation is used. Up to now, however, the presence of "neurofilaments"

in the motor endings of striated muscles has not been clearly determined, and it might be asked if these filaments are not completely absent from the terminal nerve branches. This conclusion appears, nevertheless, quite unlikely, since preparations in which the neurofibrils are stained clearly show the existence of terminal neurofibrils extending the motor axon neurofibrils beyond the preterminal constriction. The presence at the same time of two kinds of submicroscopic neurofilaments of different size and nature (Palay, 1958) in the axoplasm of the nerve fibers may explain this apparent discrepancy between the data furnished by light and electron microscopy. On the one hand it is possible that the neurofilaments of larger caliber are not prolonged in the terminal axoplasm and, on the other hand, that the neurofibrils stained by the silver methods inside the terminal nerve branches are the result of the agglutination of the other submicroscopic neurofilaments, whose existence in the nerve fibers is not always easy to demonstrate with the present methods of electron microscopy.

V. TELOGLIA

The cells accompanying the terminal branches of the motor axon, and which collectively form the teloglia, are visible with a light microscope only at the level of their nuclei (arborization nuclei), each of which is generally surrounded by a small heap of cytoplasm.

A. Arborization Nuclei

Ranvier (1878, 1888) was the first author to distinguish clearly the arborization nuclei from, firstly, those nuclei belonging to the endothelial sheath which continues the Henle's sheath above the motor end-plate, and, secondly, from the fundamental nuclei (sole nuclei of Kuhne).

Kuhne (1886, 1887, 1892) always disagreed with this distinction, and would admit only the presence of sole nuclei, and of nuclei attached to the telolemma, i.e., to the membrane coating the motor end-plate.

Kuhne was not the only one who denied the existence of a special category of nuclei bound to the terminal nerve branches. Cajal (1899, 1909), Tello (1907, 1917), Iwanaga (1925), Stöhr (1928), Cuajunco (1942), and many others, although generally admitting that the relationship of certain nuclei with the nerve end-ramifications is particularly intimate, do not consider them as quite different from the other sole nuclei. As for the origin of arborization nuclei, it was long debated.

It seems that Ranvier himself, uncertain as to the nature of the

fundamental nuclei, did not wish to commit himself fully concerning the nature of the arborization nuclei.

Rio-Hortega (1925), while he notes that the significance of these nuclei is "not very clear," regards their muscular origin as probable.

As we shall see further, the existence of the arborization nuclei, and their homology with the Schwann cell nuclei, may be very clearly established by studying the development of the motor end-plate (Couteaux, 1938a, b, 1941). The teloglia is most easily distinguished from the rest of the end-plate when it forms, together with the nerve branches, a rounded corpuscle which depresses the neighboring nuclei.

The embryonic muscle preparations, however, are conclusive in this respect only if the end-plates are seen as a whole, and have been stained by a method which brings out the differences in the structure of the end-plate nuclei, especially those concerning their nucleoli.

A study of motor end-plate development led Boeke also (1942, 1944, 1949) to distinguish nuclei of lemmoblastic origin, which become arborization nuclei, from those of muscular origin found at the level of the neuromuscular junction. But, although the silver methods he used show the neurofibrils very clearly, and stain the nuclei chromatin quite deeply, they do not always stain the nucleoli of the muscle nuclei with sufficient strength to enable them to be distinguished from other nuclei.

According to Boeke, the cells to which the arborization nuclei belong are homologous to the "interstitial cells" described at the level of the terminal plexus of the autonomous nervous system; the cytoplasm of these cells and the neighboring sarcoplasm would "merge" into the sole protoplasm.

Further research on motor end-plates by Tello (1944), using grown animals, showed that a nucleated sheath, probably prolonging the Schwann sheath at the level of the end-plate, accompanies the terminal nerve branches along their entire length.

In spite of important differences in the general interpretation of the motor end-plate, two points are agreed upon by the three above-mentioned authors: one, cells having the same origin as Schwann cells are found in the end-plate, and, two, their nuclei are those described by Ranvier as arborization nuclei.

B. Relationship between Teloglia and Terminal Axoplasm

Referring to the structure of the terminal axoplasm, it has already been pointed out that complex problems are raised by the interpreta-

tion of pictures resulting from methods which use impregnation by gold, silver, or postvital stainings. The same difficulties arise when these procedures are used to define the relationship between teloglia and nerve terminal branches.

Tello (1944) concluded that each branch of the end arborization is completely enclosed in a nucleated sheath. The axoplasm would be, as a consequence, entirely separated from the junctional sarcoplasm by this sheath, even inside the synaptic gutters.

Through comparison of aspects of the nerve ending obtained by postvital staining with methylene blue or Janus green B, and by silver methods, Couteaux (1945c, 1947) described two distinct parts in the end-bush of a frog: one, axial, extending the axon, and the other, surrounding the first, which he regarded as belonging to the teloglia. This peripheral part forms a continuous sheath around each nerve branch. Couteaux compared it to the "stroma" described by Kuhne (1887) by means of gold methods, but which the latter had considered as a component of the nerve end arborization.

This view of Kühne's stroma as a sheath made of nonnervous protoplasm was not new. Tiegs (1932) showed on reptilian muscles that the stroma (which he called "perilemma" in order to emphasize its independence from the axon) survives degeneration of the nerve after section, and remains in direct continuity with the cord of Bungner, i.e. Schwann cells of the degenerated nerve. This observation appeared to give an interesting evidence in favor of the glial nature of the Kuhne's stroma (Tiegs, 1953).

Examination of sections made through the motor end-plate after fixation by means of good osmic fixatives (Bensley, osmiochromic fluid of Laguesse), and staining by mitochondrial methods, shows that there is no room in the synaptic gutters for a periaxoplasmic layer as thick as Kuhne's stroma.

The conclusions drawn from electron microscope observations have been in this respect much more categorical, since all the researchers who have hitherto studied the structure of the end-plates with the electron microscope (Palade and Palay, 1954; Reger, 1954, 1955, 1957; Robertson, 1954, 1956) deny that any teloglial cytoplasmic layer, even of the thinnest dimensions, is present between the axoplasm and the sarcoplasm.

Due to its high resolution and the thinness of its sections, electron microscopy is much more suitable than light microscopy for solving

such a problem, and, consequently, it may be considered as established that there is no interposition at all of teloglia between the axoplasm and sarcoplasm at the level of the motor end-plate. Although the teloglia is closely tied to the nerve ending, beginning with the first stage of the formation of the motor end-plate (Couteaux, 1938a, b, 1941), it does not completely enclose the nerve ending and does not penetrate to the interior of the synaptic gutters (Fig. 4). It is possible, particularly in the frog, that slender teloglial processes sometimes form a loop around the terminal nerve branches; it seems, however, that such an interposition between the pre- and postsynaptic membranes would be practically negligible as far as the functional relationships of these two membranes are concerned.

A similar problem has been posed by interneuronal synapses. Hitherto, in every case where an examination with the electron microscope has been made, the conclusion was the same, i.e., there is no neuroglial interposition between the pre- and postsynaptic membranes.

Concerning the general arrangement of the teloglia, it seems, however, to have been previously demonstrated with the light microscope that the teloglia follows each of the branches of the nerve ending along its entire length.

Several electron micrographs by Reger (1955) show the intimate connections of the teloglia with the nerve terminals.

Robertson (1956) established, in a chameleon lizard, that above the nerve twig a thin layer of cytoplasm exists which differs decidedly in appearance from the underlying axoplasm, from which it is separated by a double membrane. Though the nature of this superficial layer of cytoplasm has not been established beyond doubt, it seems highly probable that it may be considered as an expansion of the teloglial cell. According to this hypothesis, the teloglia closes the synaptic gutter as a lid or an operculum and does not, therefore, lie between the axoplasm and the sarcoplasm, but between the axoplasm and the extracellular medium.

Further research will show whether this firm closing of the synaptic gutter by the teloglia is the rule, and a constant feature of the end-plate organization.

Now that it has been demonstrated that the axon terminal branches have no teloglia lining inside the synaptic gutters, the stroma described by Kuhne as completely enclosing each branch of the "axialbaum" and as being more granular in its peripheral part can no longer be

considered as formed uniquely by the teloglia. It also includes, without any doubt, an axoplasmic part, since Kühne describes it as existing not only around the nerve terminal branches, but also at the level of the last myelinated segment of the motor axon, inside the myelin sheath (1887, Fig. 47).

On the other hand, the structures described as stroma on the end-plates, seen in front view and on cross sections, are doubtless not the same. Several of the cross sections of the motor end-plates drawn by Kuhne show beyond doubt that, at the level of the space located between the fibrillar axis deeply stained by a gold method and the sarcoplasm, the stroma is formed, at least in its peripheral part, by the subneural apparatus.

It seems probable, therefore, that the stroma described with a gold method includes some very different structures belonging to the three tissues which enter into the make-up of the motor end-plate.

As the most recent data show that the teloglia accompanies the terminal nérve branches, although not interposed between the axoplasm and the sarcoplasm, the only part of the stroma which can be regarded as teloglial at the level of the junction is the one which is found above the nerve branches, outside the synaptic gutters.

VI. JUNCTIONAL SARCOPLASM

In mammals, the junctional sarcoplasm, together with the fundamental nuclei and its granules, generally form only a slight protuberance on the surface of the muscle fiber when the latter is not contracted. As Kuhne (1887) had already noted, however, this mass of sarcoplasm may become quite prominent if the muscle fiber is contracted.

In the frog, whose nerve branches are nearly always quite long, the junctional sarcoplasm does not form a protuberance which can be seen, even when the muscle fiber is tightly contracted. This difference between the neuromuscular junction of batrachians and those of higher vertebrates was emphasized by Rouget (1864) in a paper on motor endings, in which he pointed out also that the prominence of the "terminal cone or eminence" in the Tardigrada, described by Doyère (1840), depends above all on the "mechanical tension" of the motor nerve, and varies widely as a result with the movements of the muscle fibers.

Whether a protuberance of sarcoplasm exists or not at the neuromuscular junction, the terminal branches of the motor nerve ending

are invariably found in the surface depressions of the junctional sarcoplasm. In vertebrates, these depressions often take the form of gutters, modeled on the nerve branches.

This surface position of the motor nerve ending was observed by Gutmann and Young (1944), using silver impregnation, in lateral views of the motor end-plate of the rabbit, and by Couteaux (1944, 1945a), while studying the subneural apparatus, stained with Janus green B, in cross sections of muscle fibers of the mouse.

The nervous and muscular substances enter directly into contact at the bottom of these "synaptic gutters."

In those vertebrates whose motor end-plates have been studied in detail, it has been remarked that the sarcoplasm presents at the level of the synaptic gutters structural or cytochemical particularities which it exhibits nowhere else. On account of these particularities, the layer of sarcoplasm located immediately below the terminal nerve branches appears as a differentiated zone of the muscle fiber described as the subneural apparatus.

A. Subneural Apparatus

In the layer of subneural sarcoplasm, selective stainings show a collection of elongated ribbon-shaped lamellae attached by one edge along their entire length to the sarcoplasm membrane. These lamellae are attached along fairly equidistant lines, giving a periodic structure to the subneural apparatus.

The subneural lamellae may be stained by several methods. The first

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Plate II. Subneural apparatus. Acetylthiocholine method after fixation by formaldehyde. (a)–(e). *Hedgehog motor end-plate (intercostal muscles)*. Magnification: (a) × 2,700, front view of the end-plate with the focus on the deeper part of the subneural apparatus. Magnification. (b) × 2,700, "synaptic gutter" seen in cross section, without counterstaining (contrast of the unstained structures slightly increased by diaphragm adjustment), the subneural lamellae (folds of the electron micrographs) appear as rodlets if they are cut perpendicular to their axis; on the right, a fundamental nucleus, unstained, is hardly visible. Magnification: (c) × 4,000; (d) × 3,300, (e) × 3,700, cross sections through end-plates, with staining of the nuclei by hematoxylin, above the subneural apparatus, teloglial nuclei, and below it, fundamental nuclei (from Couteaux, 1955). (f)–(i): End-bushes of the frog (gastrocnemius); front view of the s
end-bushes. Magnification. (f) × 3,85
segment belonging to the same end-bush

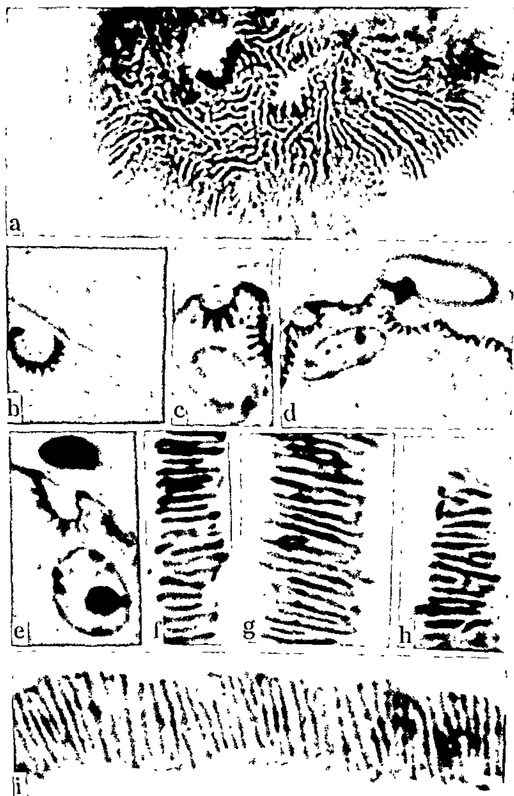


PLATE II

In reptiles and mammals, the orientation often varies considerably, depending on whether we are dealing with the deep part of the gutter or the areas adjacent to the edges.

In the deep part of the gutter, the folds may be orientated in different patterns, often longitudinally. In the latter case, the folds may be as long as the gutter itself, and the appearance of the synaptic gutter, seen on a section cut perpendicular to its axis, resembles part of a cog wheel (Plate II, b–e), the cogs corresponding to the folds seen in cross section.

Bordering the edges of each gutter, the subneural folds have, on the contrary, a fixed orientation, perpendicular to each edge. The arrangement of folds in these areas explains why the sections cut transversally to the gutter axis have no cogs near the edges, except at the gutter extremity.

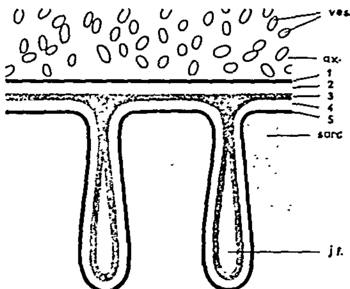


Fig. 5. Schematic drawing of two junctional folds, showing how the sarcoplasm surface membrane joins with the axoplasm surface membrane and forms the synaptic compound membrane. ax., axoplasm; ves., vesicles; sarc., sarcoplasm; j.f., junctional fold (after Robertson, 1956, slightly modified).

It is possible that the unstained zone existing in the deepest part of each infolding corresponds to the section of a canaliculus. Observations with the light microscope, moreover, invariably show, on the sides of the synaptic gutter, outcroppings of the subneural lamellae at the surface of the sole. If, therefore, the unstained zone visible inside the folds is really a "free" space, it is probable that the canaliculi of the subneural

folds open at the end-plate surface by means of slot-shaped openings placed on either side of each nerve branche (Couteaux, 1957b).

In the animal species where numerous anastomoses unite the subneural folds, the canaliculi would form a kind of network below each terminal nerve branch.

It is obvious that such a system of canaliculi may play an important part in the normal functioning of the normal motor end-plate, and may allow, moreover, the pharmacodynamic agents to reach more easily both the synapse and the junctional sarcoplasm.

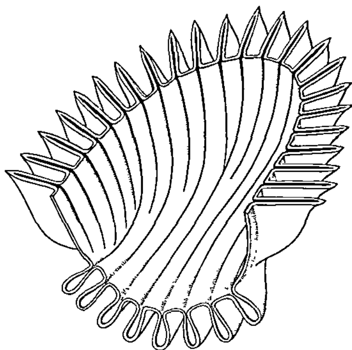


FIG. 6. Schematic three-dimensional presentation of the subneural apparatus at the extremity of a "synaptic gutter." This interpretation is based upon data hitherto obtained with both the light and the electron microscope (cf. Fig. 4).

It may be assumed that the canaliculi bring into relationship the diverse regions of the postsynaptic membrane, including even those regions located in the deepest part of the gutters, and the extra-cellular medium.

The folds are often prolonged at the surface of the end-plate by other canaliculi which correspond to the superficial threads sometimes quite long, observed with the light microscope.

The study of the submicroscopic structure of the subneural apparatus has only just begun, but it is already certain that this layer of sarcoplasm, having a thickness of about one micron, and limiting the synaptic

gutters, presents morphological features of a highly specialized zone.

Let us now consider to what degree the sarcoplasmic membrane in the region of the subneural infoldings differs from that of the remaining muscle fiber.

Postvital methods allow the selective staining, on the surface of the striated muscle fiber, of a component of the sarcolemma which appears, with the light microscope, as a continuous and homogeneous membrane (Couteaux, 1947). This membrane extends under the terminal nerve branches at the motor end-plate, and is prolonged by the folded membrane of the subneural apparatus.

It is probable that the membrane stained by the periodic acid-Schiff method (PAS reaction) at the level of the sarcolemma by MacManus (1948) is identical with the one stained by postvital methods. These staining methods reveal no important difference between the membrane coating the sarcoplasm within the synaptic area and the one enveloping the rest of the muscle fiber.

Differences are apparent between the two zones of the sarcoplasm surface membrane when other methods are used, such as Heidenhain's Azan method which stains the collagen fibrils of the endomysium, or Laidlaw's silver technique, which stains the argyrophil fibrillar network very deeply at the surface of the muscle fiber. None of these procedures bring to light the existence of any fibrillar structure at the surface of the subneural sarcoplasm. Nor has it been possible to detect collagen fibrils at this level with the electron microscope.

Further data have resulted from observations made with the electron microscope after osmic fixation.

There is a continuity between, on the one hand, the three layers of the subneural sarcoplasmic membrane (made up of a very dense inner layer, an outer layer less dense and thicker than the former, and, between these two layers, a light layer), and, on the other hand, the three corresponding layers of the sarcolemma (Figs. 4 and 5). Robertson (1956), however, remarked that the total thickness of these three layers is slightly less at the level of the synaptic gutters, due mainly to the thinning out of the light middle layer. An interpretation of this fact would at present be premature.

Histochemical methods which reveal cholinesterase activity have proved, up to the present moment, to be the most effective ones for clearly defining the differences between the sarcoplasm membrane located within the synaptic area and the remainder of this membrane. These

procedures disclose at the motor end-plate an important concentration of cholinesterase, mainly located at the postsynaptic membrane.

The location of cholinesterase at this point is quite typical of the subneural apparatus. In vertebrates, this cytochemical characteristic seems to be a more constant attribute of the subneural differentiation of the sarcoplasm than the presence of folds, which appear to be wanting at some neuromuscular junctions, for example on frog's slow muscle fibers and teleost's muscle fibers. The question of enzymatic location will be discussed later in more detail.

Further investigation will be necessary not only to complete the study of the sarcoplasmic membrane at the level of the synaptic gutters, but also to examine the particularities of the thin sarcoplasm layers located between the folds.

Since it appears probable that the deep part of the subneural apparatus rather far distant from the axoplasmic membrane does not play exactly the same role as the properly synaptic part of this apparatus in the transmission of the excitation from the motor nerve to the muscle, a more thorough morphological and cytochemical investigation will perhaps reveal significant differences between the two parts.

B. Relationship between Axoplasmic and Sarcoplasmic Membranes

Due to the existence of the subneural infoldings, the sarcoplasm surface membrane presents at the end-plate two parts having very different relationship with the axoplasm surface membrane. One is closely joined to the axoplasm surface membrane, whereas the other corresponds to the infoldings at the level of which the membrane limiting the junctional sarcoplasm leaves the one limiting the terminal axoplasm.

Between the infoldings, the surface membranes of the nerve fiber and muscle fiber are closely united to each other, and their connections are, in this respect, comparable with those existing between the pre- and postsynaptic membranes of a central synapse (Palade and Palay, 1954; Palay, 1956), and of a ganglionic synapse (De Robertis and Bennett, 1954, 1955; Taxi, 1957, 1958), where there is also an apposition of the two membranes. Besides, however, the junctional folds—nothing comparable to them has hitherto been noted at interneuronal synapses—the pre- and postsynaptic membranes present at the end-plate a somewhat different structure from that of the membranes forming the synaptolemma of interneuronal synapses.

At the point where the two membranes are apposed and form a synaptic compound membrane (Fig. 4), it is difficult to determine exactly what belongs to each of the two membranes. The junctional fold, however, formed solely by the sarcoplasm surface membrane, offers particularly favorable conditions for the analysis of the latter. In the chameleon lizard, Robertson (1956) describes three layers: a very dense inner layer less than 100 A. thick; an outer layer about 200 A. thick, which is also dense, but less dense than the former; and between these two layers a light layer about 100 to 200 A. thick.

As one proceeds from the junctional folds, their three layers may be seen *extending into the synaptic compound membrane which results* from the apposition of the axoplasmic and sarcoplasmic surface membranes.

The surface membrane of the axoplasm, like that of the sarcoplasm, contains a very dense inner layer, less than 100 A. thick. It seems probable that the axoplasm surface membrane also includes a light layer and a dense outer layer.

The synaptic membrane complex, about 500 to 700 A. thick, resulting from apposition, comprised five layers (Fig. 5). The two very dense inner membranes, limiting the axoplasm and sarcoplasm respectively, are separated from each other by three other layers: a dense middle layer and two light layers. One of the light layers obviously corresponds to the light layer of the sarcoplasm surface membrane, and the dense middle layer probably results from a fusion of the two dense outer layers of the axoplasm and sarcoplasm membranes[1].

Although the axoplasmic and sarcoplasmic membranes are only apposed, it is quite difficult to separate them from each other. If a strong traction is applied, after fixation, on the motor axon segment immediately adjacent to the end-plate, it is possible to detach the entire nerve ending from the muscle fiber, but, at the same time, the subneural apparatus is carried with it (Couteaux, 1952, Plate II, B.). Consequently, the sarcolemma breaks off around the end-plate, with-

[1] This description given by Robertson of the synaptic membrane complex was based for the most part upon OsO_4-fixed, phosphotungstic acid-stained preparations. Later investigations led Robertson (1957, 1958) to consider as plasma membranes only the very dense layers next to axoplasm and sarcoplasm. Each of these plasma membranes would be made up of two dense layers 25 A. thick separated by a light layer of about the same width The rest of the synaptic membrane complex would be extracellular, perhaps comparable to a basement membrane, and would probably represent a highly hydrated gel

out the axoplasmic membrane separating from the sarcoplasmic membrane. This phenomenon can doubtless be explained, in large part, by the ties, *in particular fibrillar, uniting the coating of the motor end-plate* to that of the motor axon; in all likelihood, it is also a result of the strong adherence, at least after fixation, of the axoplasmic and sarcoplasmic membranes at their junction.

C. Fundamental Nuclei and Inclusions of the Junctional Sarcoplasm

As the study of the development of motor end-plates and the delimitation of the sarcoplasm in adult end-plates (Plate II, c–e) have established, the sole nuclei or fundamental nuclei belong to the muscle fiber. For quite a long time, they have been described as large, clear, rounded nuclei, containing one or two rather bulky nucleoli.

The shape of the fundamental nuclei often contrasts with that of ordinary muscle nuclei, the latter often being elongated, with their axes disposed parallel to the direction of the myofibrils. This difference serves as one of the premises of those authors who do not consider the fundamental nuclei and the surrounding granular cytoplasm as muscular, attributing to them the same origin as the Schwann cells (Iwanaga, 1925; Noel, 1927, 1950).

In fact, only the "sarcoplasm-poor fibers" nuclei exhibit a marked difference; these fibers make up the greater part of the "white muscles," where the ordinary muscle nuclei are surrounded only by a very small quantity of sarcoplasm. The difference in shape of nuclei occasionally disappears completely when it is a question of "sarcoplasm-rich fibers" having an accumulation on their surface of an important quantity of peripheral sarcoplasm distributed mainly around their nuclei.

It appears that the muscle nuclei—the ordinary muscle nuclei as well as the fundamental nuclei—invariably present a rounded form when they are located inside a considerable mass of sarcoplasm, as if they were no longer subject to the direct mechanical action of the myofibrils, with the resulting deformations. As a consequence, the form of the fundamental nuclei would depend above all on the quantity of sarcoplasm surrounding them, and would not be an intrinsic feature distinguishing them from ordinary muscle nuclei. Moreover, because the form and size of the nucleoli are quite variable, one cannot distinguish the fundamental nuclei from the other muscle nuclei on the basis of the structural differences of the nucleoli.

membrane of a type similar to those of the Schwann cells and of the muscle fiber.

For a long time, it has been known that collagen fibrils are found adherent to the endothelium and strengthening it. Many collagen fibrils are also present in the space between this sheath and the surface of the end-plate.

The relationship between the sarcolemma and the nerve terminal branches has been the subject of lively controversy since the first investigations on the motor end-plate. The final conclusion was that the nerve terminal arborization is located below the sarcolemma, i.e. hypolemmal.

The above description of the synaptic gutters, where the axoplasm of the terminal nerve branches is separated from the sarcoplasm not only by the axoplasmic membrane, but also by a membrane prolonging the sarcolemma, showed that at present it is not possible to consider the nerve ending as hypolemmal in the usual sense of the word, i.e. as located below the sarcolemma. Whether or not the nerve branches are found deeply inside the sarcoplasm, the sarcoplasm surface membrane always passes below them, and presents the same general structural pattern at this level as at all other points of the sarcoplasm surface. One might conclude from this fact that the nerve ending is located above the sarcolemma, and that therefore it is epilemmal, but this term is not exactly appropriate. The sarcoplasm surface membrane seen below the nerve terminal twigs does not, in fact, seem to possess a fibrillar lining, and may not, therefore, be considered as quite the same as ordinary sarcolemma.

The discarding of the terms, used hitherto, of "hypolemmal" and "epilemmal," has already been proposed (Couteaux, 1947). These terms are today even more out-of-date, since they refer chiefly to a morphological distinction between motor and sensory endings, a distinction not confirmed by electron microscopy investigations.

VIII. LOCALIZATION OF THE JUNCTIONAL CHOLINESTERASE

A study of the distribution of cholinesterase activity at the neuromuscular junction has been undertaken subsequent to the work of Dale and Feldberg (1934); Dale *et al.* (1936); Brown *et al.* (1936), who had shown that the transmission of excitation from motor nerve to striated muscle is accompanied by a release of acetylcholine. Its main object was to determine whether the cholinesterase, which exists in a weak

concentration in nearly all animal tissues, has at the end-plate a concentration which is high enough to ensure that the acetylcholine released at each nerve impulse is destroyed during the refractory periods, i.e. in a few milliseconds.

The first research on the distribution of activity in the striated muscle was carried out on the sartorius of the frog (Marnay and Nachmansohn, 1937) and toad (Feng and Ting, 1938). Manometrically, this research established that hydrolysis is more rapid in that region of the muscle where motor nerve endings are located than in other regions of the muscle, and more rapid than at the level of the motor nerve itself.

Concerning mammalian muscles, a few manometric determinations on the gastrocnemius of the dog corroborated results obtained with batrachians, but the first really significant data were obtained only later, on the m. gastrocnemius internus of the guinea pig (Couteaux and Nachmansohn, 1940, 1942).

As the motor end-plates represent only an extremely small fraction of the total volume of the muscle, it was inferred from the differences in enzymatic activity observed between muscle regions rich in, or deprived of motor end-plates, that enzymatic concentration is probably extremely high at the level of the motor end-plate itself. This conclusion was subsequently verified histochemically for the first time by Koelle and Friedenwald (1949) and Koelle (1950) with the thiocholine method on the muscles of the rat.

Once it was established that the cholinesterase is more highly concentrated at the level of the neuromuscular junctions than at the level of the motor nerve and the "aneural" part of the muscle, it still had to be determined in exactly what part of the junctional area this large amount of enzyme was located.

One opinion already put forward was that the junctional cholinesterase was probably located in the nerve endings. A study of the variations in the cholinesterase content of the striated muscle after section of the motor nerve and the consequent disappearance of the axoplasmic component of the end-plate has, on the contrary, shown that the greater part of the junctional cholinesterase long survives degeneration of the axon, and therefore is located not in the interior of the nerve endings, but outside them (Couteaux and Nachmansohn, 1938, 1940, 1942).

Histochemical methods later confirmed this interpretation. The re-

maining problem was to find out which of the various motor end-plate components is the site of this cholinesterase activity located outside of the nerve endings. By comparing several experimental and cytochemical data, the assumption was made that the junctional cholinesterase is mainly located at the level of the subneural apparatus (Couteaux, 1947).

The histochemical pictures obtained by most authors (Couteaux and Taxi, 1951, 1952; Couteaux, 1951; Holt and Withers, 1952; Coers, 1953a, b, 1955; Gerebtzoff, 1953; Denz, 1953; Gerebtzoff *et al.*, 1954; Harris, 1954; Holt, 1954; Woolf and Till, 1955; Kovac *et al.*, 1955; Crevier and Belanger, 1955) showed that the structures selectively stained are those which have been described as the subneural apparatus (Plate II).

With thiocholine methods, using acetylthiocholine and butyrylthiocholine as substrates, and when the pH and buffers have been appropriately chosen, the subneural apparatus appears as the main site of the cholinesterase activity of the end-plate.

The appearance at pH values of important diffusion artifacts, entailing the staining of other structures, challenges the validity of this localization. It has been proved that enzyme diffusion may be practically disregarded, and that only the thiocholine diffusion should be taken into account.

By mechanical separation of the subneural apparatus from the other juxtasynaptic components before incubation (Couteaux, 1958), and by a histochemical study of the cholinesterase activity of the denervated end-plates, it has been possible to establish that selective staining of the subneural apparatus is not the result of a thiocholine diffusion from a neighboring structure.

Histochemical observations have already been made repeatedly on the cholinesterase activity of denervated end-plates. Sawyer and associates (1950), in a preliminary report, and Kupfer (1951) indicate for the rat, the guinea pig, and the rabbit, persistence of cholinesterase concentration at the level of motor end-plates after section of motor nerve and degeneration of nerve endings. Subsequently, after a more thorough histochemical study, Coers (1953c), Snell and MacIntyre (1955, 1956), and Savay and Csillik (1956) arrive at the same result. These authors have shown that the cholinesterase activity which persists in the denervated end-plate of the rat and the guinea pig is in this case also located at the level of the subneural apparatus. The whole appear-

ance of this apparatus generally altered 2 weeks after the section of the motor nerve, but at this stage, the lamellae may still be seen and stained by histochemical methods.

Appreciable differences are shown between figures hitherto published regarding the time limit for persistence of cholinesterase activity in the denervated end-plates of the rat and the guinea pig. Three months after the section of a motor nerve, Coërs still found, in the gastrocnemius of the rat, some end-plates which showed a deformed, faintly stained, but easily recognizable subneural apparatus. In the same species, and on the same muscle, Savay and Csillik noted a residual activity after 4 months, and even after 6 months; while with the guinea pig, Snell and MacIntyre found no activity after 45 days.

It would seem probable that these differences are mainly due to minor differences in the technique used. With Koelle's technique, and checking for the absence of nerve regeneration, Carric (unpublished results) observed, on certain greatly altered motor end-plates of the m. gastrocnemius internus of the rat and guinea pig, the persistence of subneural lamellae faintly stained but still visible 6 weeks after section of the motor nerve.

In any case, conclusions reached today from histochemical research on the cholinesterase activity of the denervated end-plates prove that the staining of the subneural apparatus by means of the thiocholine method is not the result of a diffusion from the terminal nerve branches.

Histochemical methods for localizing cholinesterases using substrates other than acetylthiocholine and butyrylthiocholine, such as the long-chain fatty acid esters, for example myristoylcholine (Denz, 1953), and α-naphthyl acetate (Denz, 1953), indoxyl derivatives (Holt, 1954), and thiolacetic acid (Crevier and Belanger, 1955) also selectively stain the subneural apparatus.

After the investigations made by Denz (1953, 1954) and Holmstedt (1957a, b), with the aid of several substrates and a number of selective inhibitors of cholinesterases, it seems that the end-plate contains two cholinesterases, an acetylcholinesterase and an unspecific cholinesterase the second of these being present in a much smaller quantity than the first.

Before electron microscopy enabled us to conceive of the subneural apparatus in ultrastructural terms, the possibility that a thin teloglial layer was incorporated in the subneural lamellae, and survived degeneration of the nerve endings after section of the motor nerve, could

not be excluded. Neither, therefore, did it seem possible to exclude completely the possibility that a cholinesterase attached to the subneural apparatus might be located in teloglia.

As a result of electron microscope examination, the subneural apparatus may now be considered as formed exclusively by the sarcoplasm surface membrane. We must therefore conclude that the cholinesterase activity observed at the level of the subneural apparatus, after denervation of the end-plate, is exclusively located at the level of the sarcoplasm surface membrane.

As, at normal end-plates, the terminal axoplasm membrane, i.e. the presynaptic membrane, is intimately apposed to the postsynaptic membrane, it is impossible to distinguish one from the other by the use of the light microscope alone, when localizing the cholinesterase. Further histochemical research with the electron microscope will therefore be necessary before deciding whether or not a cholinesterase site exists at the level of the presynaptic membrane. In any case, results obtained from the above-mentioned biochemical investigations on denervated muscle show that the cholinesterase activity at a possible presynaptic site is incomparably smaller than the activity located in the subneural part of the postsynaptic membrane.

Based upon biochemical data, it may already be admitted that outside the subneural lamellae there exists a certain amount of cholinesterase, as the "aneural" part includes a noticeable part of the total quantity of enzyme contained by the muscle. The failure to find the site of this moiety of the enzyme by the acetylthiocholine method is not surprising, since this method is not very sensitive.

Lastly, Zacks and Welsh (1951) showed that unspecific and specific cholinesterases are present in rat liver mitochondria. We have consequently to admit as possible a similar location at the level of the muscle mitochondria, and particularly of the numerous mitochondria of end-plates.

IX. MORPHOGENETIC SIGNIFICANCE OF THE MOTOR END-PLATE

Insofar as the terminal axoplasm and the teloglia are concerned, the preceding description of the different components of the motor end-plate does not bring to light any characteristics essentially different from those observed at the level of the central and ganglionic synapses.

Such is not the case for the structure of the junctional sarcoplasm, i.e. the postsynaptic component of the motor end-plate, and its relation-

ship with the terminal axoplasm. In general, at an interneuronal synapse, the postsynaptic membrane is simply apposed to the presynaptic membrane, forming a double membrane; the postsynaptic axoplasm, at least up to now, seems not to present any really significant particularity in the juxtaterminal area. The existence of the subneural apparatus at the motor end-plate clearly shows, on the contrary, the existence of a quite special organization of the postsynaptic sarcoplasm at the neuromuscular junction.

The study of the motor end-plate development allows the observation of the genesis of this differentiation, and suggests assumptions regarding its mechanism. Two main phases may be distinguished in the evolution of neuromuscular relations during embryonic development in mammals.

In the first phase, the muscle fibers are still in "myotube" form, with axial nuclei and peripheral myofibrils. The motor nerve fibers, still few in number, proceed through the muscle, thus clearing the first motor intramuscular pathways. These are the "exploring" nerve fibers, whose extremities have as yet developed no lasting connections with the muscle fibers. Even when they are closely apposed to the myotubes, no significant reaction thus far discernible from the sarcoplasm and muscle nuclei is provoked by these "free" motor endings situated at their level.

It is in the second phase that the final junction of the motor nerve fibers and muscle fibers is established, when the muscle nuclei, originally axial, have completed their migration to the surface of the myofibrillar bundle. Once the margination of the muscle nuclei is finished, the nerve fibers send out either terminal or lateral sprouts, generally accompanied by Schwann cell nuclei, and each of these sprouts enters into close relations with a muscle fiber at the level of one of its nuclei, now peripheral.

At the contact of the nerve sprout, the muscle nucleus divides repeatedly, while at this level the sarcoplasm becomes more abundant (Fig. 7).

These divisions of muscle nuclei are generally preceded by nucleolar bipartition, and appear in all respects comparable to the amitoses described by Naville (1922), in the course of muscular development of frogs.

On the preparations of muscles stained by means of the Bielschowsky-Gros method, the nucleoli of the muscle nuclei appear very clearly, and it is not difficult to find, in the vicinity of the nerve sprouts, the characteristic aspects of nucleolar bipartition. What is most frequently found in the muscle nuclei during the process of division are enlarged nucleoli,

Not only does the study of the setting up of neuromuscular connections enable the main components of the motor end-plate to be defined, but it also brings to light some of the interactions which occur when a junction is being formed.

From an analysis of the first stages of the process leading to the formation of the motor end-plate, one fact stands out clearly: to effect the junction of a muscle fiber and nerve fiber from which a motor end-plate will result, it is not sufficient that these fibers should come into direct contact with each other; it is also necessary for one, or perhaps both of these fibers, to have reached a certain stage of their development. It is, indeed, at a relatively late period of the muscle development, when the nerve fibers have already been in contact with the embryo muscle fibers for some time, that the first outline of the final connections appears.

These connections are established only after the margination of the muscle nuclei, and always at their level, at least in mammals (London and Pesker, 1906; Tello, 1917; Couteaux, 1941). It seems probable that the muscle nuclei, or the sarcoplasm nucleated zones, intervene directly in the junctional process by exerting an attraction on the neighboring neurites.

At the moment of this attraction, the axon which crosses a muscle fiber is never very far from one of the nuclei which protrude at the myofibrillar bundle surface; thus the attraction always comes into play at a short distance. It determines the formation of a short nerve sprout, and the nearer the axon from which it originates to a muscle nucleus, the shorter the nerve sprout.

The mechanism of this attraction exerted by muscle fiber nucleated zones on the motor axons is still not well understood.

Its intervention marks the beginning of a new phase in neuromuscular relations and ends the "free" growth phase of the motor nerve fibers, during which the growth orientation appears to be governed by the "contact guidance," as defined by Weiss (1941).

The study of the motor end-plate development shows that the accumulation of muscle nuclei and sarcoplasm observed at the end-plate is not, as believed in the past by several authors, antecedent to the junction, but results from this junction.

The multiplication of the muscle nuclei at the point of contact of the motor nerve sprout and the muscle fiber differs, in at least one respect, from the nuclear multiplication observed over the whole length of the muscle fiber during its growth; as a rule, during muscle development,

muscle nuclei resulting from a nuclear division tend to draw away from one another as if by a *mutual repulsion; in the case of divisions which* take place at junction level, they remain juxtaposed. Thus there is formed at each junction a cluster of muscle nuclei, and, correlatively, *an* accumulation of sarcoplasm which can be regarded as due to local effects caused by contact of the motor nerve sprout with the muscle fiber.

The formation of the subneural apparatus also appears to be a local effect due to the influence of the embryonic motor nerve ending on the *muscle fiber.*

When the striated muscle fibers of a guinea pig, removed little before its birth (e.g. from an 80 mm. embryo) are postvitally stained with Janus green B, the sarcoplasmic membrane, at the level of the embryonic motor end-plate, is depressed by the nerve ending, the latter being *surmounted by one or two teloglial nuclei.*

The thiocholine method shows that a cholinesterase activity is present at the level of this subneural membrane, which, observed with the light microscope, appears thicker than the sarcoplasmic membrane of other regions of the muscle fiber (Plate III, b, d, e).

This thickening is easily explained by the formation of subneural lamellae, which observations with the electron microscope on adult mammals reveal as infoldings of the sarcoplasmic membrane.

On the muscles of a newborn guinea pig, these subneural lamellae —very close-set and not yet penetrating deeply into the sarcoplasm— *are clearly perceptible with the light microscope, after staining either* with Janus green B or by the thiocholine method.

The configuration of the subneural apparatus at each stage of the developing motor end-plate depends directly on the form of the nerve ending at this same stage. Immediately following the junction of the *motor nerve fiber and the muscle fiber, it takes the form,* in the guinea pig, of a shallow cupule, whose edges are level with the surface of the junctional protuberance; the concavity, however, of this primary subneural apparatus is sometimes extremely slight.

The subneural cupule subsequently widens, correlatively with the development of the *nerve ending, which extends to the surface* of the muscle fiber and branches out.

Under the pressure of the terminal nerve branches, whose dimensions increase, the subneural apparatus which heretofore was of simple form and presented an almost regular curve, gradually acquires a lobed



PLATE III

outline and complicated relief. Gutters appear under the nerve ending branches, constituting the first outlines of adult synaptic gutters. From this moment, the subneural lamellae reach the surface of the junctional protuberance on both sides of each nerve branch.

During the course of postnatal growth, these gutters, which are molded on the nerve branches, widen and lengthen at the same time as the branches. In the guinea pig, they become much deeper, but, as has already been mentioned in connection with the description of the subneural apparatus, the final depth of the synaptic gutters is quite variable, depending on the animal species. Many other specific morphological differences accompany these variations in depth.

The formation of the subneural apparatus, characterized by special folds and acetylcholinesterase activity, clearly results from an inductive action exerted directly by the nerve ending on the subneural sarcoplasm.

If one compares the local influences at work on the sarcoplasm exerted by the motor nerve endings and the sensory nerve endings, respectively, during the formation of the motor end-plates and the neuromuscular spindles, it will be seen that they both entail the formation of a mass of muscle nuclei in their immediate vicinity. However, one may observe *two important differences between them: the sensory* nerve endings of the neuromuscular spindles do not determine, as do the motor nerve endings, the formation of a subneural apparatus at the level of the junctional sarcoplasm; further, they generally exercise on

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PLATE III. Neuromuscular junctions of 80–85 mm. guinea pig embryos (m. latissimus dorsi). (a): Silver preparation. Three nuclei may be observed at the level of this embryonic neuromuscular junction: one teloglial nucleus, accompanying the nerve ending, and two muscle nuclei. Magnification: × 1,550 (from Couteaux, 1941). (b): Acetylthiocholine method after fixation by formaldehyde, with staining of the nuclei by hematoxylin. Lateral view of two neuromuscular junctions. At this stage of the development, the subneural apparatus assumes the form of a shallow cupule, in which lie the nerve ending and the teloglia. Magnification: × 1,620. (c). Silver preparations. The muscle nuclei, already numerous, form an arc of a circle around the nerve ending and the teloglia. Magnification: × 1,070 *(from* Couteaux, 1941). (d) and (e): acetylthiocholine method after fixation by formaldehyde, with staining of the nuclei by hematoxylin. (d): Magnification: × 1,680: front view of a neuromuscula
section, appears as a curved
muscle nuclei cluster. (e): M.
junction. Three kinds of nuclei are visible: below the subneural apparatus, several muscle nuclei; above it two teloglial nuclei, surmounted by one nucleus belonging to the endothelial coating of the junction.

the myofibrillogenesis a local inhibitory action which the motor nerve endings never seem to exercise at the neuromuscular junctions.

Since the presynaptic nerve endings of the ganglionic and central synapses are in several respects homologous to the terminals of the motor axons innervating the striated muscle, one may ask if at the level of these synapses the nerve endings do not exert on the "subsynaptic" zones of the nerve cell a morphogenetic influence comparable in some degree to that which determines the formation of the subneural apparatus at the level of the junctional sarcoplasm of the muscle fiber. The morphological and cytochemical methods have not up to the present moment provided data which allow one to consider the subsynaptic zones of the nerve cell as specialized areas; however, in the present state of these methods, the negative results which they furnish cannot be held as conclusive.

References

Boeke, J. (1909). *Anat. Anz.* **35**, 192.
Boeke, J. (1911). *Intern. Monatschr. Anat. Physiol.* **28**, 376.
Boeke, J. (1927). *Z. mikroskop. anat. Forsch.* **8**, 561.
Boeke, J. (1932). *In* "Cytology and Cellular Pathology of the Nervous System" W. Penfield, ed.), Vol. 1, p. 243. Hoeber, New York.
Boeke, J. (1942). *Koninkl. Ned. Akad. Wetenschap. Proc.* **45**, 444.
Boeke, J. (1944). *Acta Neerl. Morphol.* **5**, 189.
Boeke, J. (1949). *Acta Anat.* **8**, 18.
Boeke, J., and Noel, R. (1925). *Compt. rend. soc. biol.* **92**, 263.
Brown, G. L., Dale, H. H., and Feldberg, W. (1936). *J. Physiol. (London)* **87**, 394.
Cajal, S. R. y (1899). "Textura del sistema nervioso del Hombre y de los Vertebratos." N. Moya, Madrid.
Cajal, S. R. y (1904). *Trabajos Lab. Invest. Biol. Madrid.* **3**, 97.
Cajal, S. R. y (1909). "Histologie du système nerveux de l'Homme et des Vertébrés," Vol. I. Maloine, Paris.
Cajal, S. R. y (1925). *Trabajos Lab. Invest. Biol. Madrid* **23**, 245.
Coers, C. (1953a). *Arch. biol (Liège)* **64**, 133.
Coers, C. (1953b). *J. Belge Pathol. Med. exptl.* **22**, 306.
Coers, C. (1953c). *Acad. roy Belg. Classe sci. Mém.* **39**, 447.
Coers, C. (1955). *Acta Neurol. Psychiat. Belg.* **55**, 741.
Couteaux, R. (1938a). *Compt. rend. soc. biol.* **127**, 218.
Couteaux, R. (1938b). *Compt. rend. soc. biol.* **127**, 571.
Couteaux, R. (1941). *Bull. biol. France et Belg.* **75**, 101.
Couteaux, R. (1944). *Compt. rend. soc. biol* **138**, 976.
Couteaux, R. (1945a). *Compt. rend* **220**, 567.
Couteaux, R. (1945b) *Compt rend. soc. biol.* **139**, 376.
Couteaux, R. (1945c). *Compt rend. soc. biol.* **139**, 641.
Couteaux, R. (1947). *Rev. can. biol.* **6**, 563.
Couteaux, R. (1951). *Arch intern. physiol.* **59**, 52.

Couteaux, R. (1952). *In* "Le Muscle, Etude de Biologie et de Pathologie" (International Symposium, Royaumont, France, 1950; C. C. I. C. M. S., ed.), p. 173. L'Expansion Scientifique, Paris.
Couteaux, R. (1955). *Intern. Rev. Cytol.* **4**, 335.
Couteaux, R. (1957a). *In Proc. Electron Microscopy Conf. Stockholm, 1956* p. 188.
Couteaux, R. (1957b). *In* "Microphysiologie comparée des éléments excitables" (International Symposium, Paris, 1955; C. N. R. S., ed.) p. 255. Centre National de la Recherche Scientifique, Paris.
Couteaux, R. (1958). *Exptl. Cell Research* Suppl., **5**, 294.
Couteaux, R., and Nachmansohn, D. (1938). *Nature* **142**, 1481.
Couteaux, R., and Nachmansohn, D. (1940). *Proc. Soc. Exptl. Biol. Med.* **43**, 177.
Couteaux, R., and Nachmansohn, D. (1942). *Bull. biol. France et Belg.* **76**, 14.
Couteaux, R., and Taxi, J. (1951). *Compt. rend. Assoc. Anat.* **70**, 1030.
Couteaux, R., and Taxi, J. (1952). *Arch. anat. microscop. morphol. exptl.* **41**, 352.
Crevier, M., and Belanger, L. F. (1955). *Science* **122**, 316.
Cuajunco, F. (1942). *Carnegie Inst. Contribs. Embryol.* **30**, 127.
Dale, H. H., and Feldberg, W. (1934). *J. Physiol. (London)* **81**, 320.
Dale, H. H., and Feldberg, W., and Vogt, M. (1936). *J. Physiol. (London)* **86**, 353.
Denz, F. A. (1953). *Brit. J. Exptl. Pathol.* **34**, 329.
Denz, F. A. (1954). *Brit. J. Exptl. Pathol.* **35**, 459.
De Robertis, E. D. P., and Bennett, H. S. (1954). *Federation Proc.* **13**, 35.
De Robertis, E. D. P., and Bennett, H. S. (1955). *J. Biophys. and Biochem. Cytol.* **1**, 47.
Dogiel, A. S. (1890). *Arch. mikroskop. Anat. u. Entwicklungsgesch.* **35**, 305
Doyère, L. (1840). *Ann. sci. nat. Zool.* [2] **14**, 269.
Emmert, F. C. (1836). "Ueber die Endigungsweise der Nerven in den Muskeln nach eigenen Untersuchungen." Jenni, Bern.
Feist, B. (1890). *Arch. Anat. u. Physiol. Anat. Abt.* Jahrg. 1890, p. 116.
Feng, T. P., and Ting, Y. C. (1938). *Chinese J. Physiol.* **13**, 141.
Foettiger, A. (1880). *Arch. biol. (Liège)* **1**, 279.
Gerebtzoff, M. A. (1953). *Acta Anat.* **19**, 366.
Gerebtzoff, M. A., Philippot, E., and Dallemagne, M. J. (1954). *Acta Anat.* **20**, 234.
Gutmann, E., and Young, J. Z. (1944). *J. Anat.* **78**, 15.
Harris, C. (1954). *Am. J. Pathol.* **30**, 501.
Held, H. (1897). *Arch. Anat. u. Physiol. Anat. Abt.* Jahrg. 1897, 204.
Hines, M. (1927). *Quart. Rev. Biol.* **2**, 149.
Hinsey, J. C. (1934). *Physiol. Revs.* **14**, 514.
Holmstedt, B. (1957a). *Acta Physiol. Scand.* **40**, 322.
Holmstedt, B. (1957b). *Acta Physiol. Scand.* **40**, 331.
Holt, S. J. (1954). *Proc. Roy. Soc.* **B142**, 160.
Holt, S. J., and Withers, R. F. J. (1952). *Nature* **170**, 1012.
Hoyle, G. (1957). "Comparative Physiology of the Nervous Control of Muscular Contraction." Cambridge Univ. Press, London and New York.
Iwanaga, I. (1925). *Mitt. allgem. Pathol. u. pathol. Anat. Japan* **2**, 257.
Koelle, G. B. (1950). *J. Pharmacol. Exptl. Therap.* **100**, 158.
Koelle, G. B., and Friedenwald, J. S. (1949). *Proc. Soc. Exptl. Biol. Med.* **70**, 617.
Kovac, M., Kraupp, O., and Lassmann, G. (1955). *Acta Neuroveget. (Vienna)* **12**, 329.
Krause, W. (1863). *Z. rationelle Med.* **18**, 136.
Kuhne, W. (1862). "Ueber die peripherischen Endorgane der motorischen Nerven." Engelmann, Leipzig.
Kuhne, W. (1864). *Arch. pathol. Anat. u. Physiol. Virchow's* **29**, 433.
Kuhne, W. (1883). *Z. Biol.* **19**, 501.
Kuhne, W. (1886). *Verhandl. nat.-med. Ver. Heidelberg* **3**, 277.

Kuhne, W. (1887). *Z. Biol.* **23**, 1.
Kuhne, W. (1892). *Verhandl. d. nat. -med. Ver. Heidelberg* **4**, 1.
Kulchitsky, N. (1924). *J. Anat.* **59**, 1.
Kupfer, C. (1951). *J. Cellular Comp. Physiol.* **38**, 469.
Lawrentjew, B. J. (1928). *Z. mikroskop. anat. Forsch.* **13**, 388.
London, E. S., and Pesker, D. J. (1906). *Arch. mikroskop. Anat. u. Entwicklungsgesch.* **67**, 303.
MacManus, J. F. A. (1948). *Stain Technol.* **23**, 99.
Marnay, A., and Nachmansohn, D. (1937). *Compt. rend. soc. biol.* **125**, 41.
Naville, A. (1922). *Arch. biol. (Liège)* **32**, 37.
Noel, R. (1925). *Bull. histol. appl. et tech. microscop.* **2**, 124.
Noel, R. (1927). *Bull. histol. appl. et tech. microscop.* **4**, 382.
Noel, R. (1950). *Biol. méd. (Paris)* **39**, 273.
Palade, G. E., and Palay, S. L. (1954). *Anat. Record* **118**, 335.
Palay, S. L. (1956). *J. Biophys. Biochem. Cytol.* **2**, *Suppl.*, 192.
Palay, S. L. (1958). personal communication.
Quatrefages, A. de (1843). *Ann. sci. nat. Zool.* [2] **19**, 299.
Ranvier, L. (1878). "Leçons sur l'Histologie du Système nerveux." Savy, Paris.
Ranvier, L. (1888). "Traité technique d'histologie," 2nd ed. Savy, Paris.
Reger, J. F. (1954). *Anat. Record* **118**, 344.
Reger, J. F. (1955). *Anat. Record* **122**, 1.
Reger, J. F. (1957). *Exptl. Cell Research* **12**, 662.
Renaut, J. (1899). "Traité d'histologie pratique," Vol. 2, Pt 2, p. 972. Rueff, Paris.
Retzius, G. (1892). *Biol. Untersuch.* [*N. F.*] **3**, 41.
Rio-Hortega, P. del (1925). *Compt. rend. soc. biol.* **92**, Suppl., 3.
Robertson, J. D. (1954). *Anat. Record* **118**, 346.
Robertson, J. D. (1956). *J. Biophys. Biochem. Cytol.* **2**, 369.
Robertson, J. D. (1957). *J. Physiol. (London)* **40**, 58P.
Robertson, J. D. (1958). *J. Biophys. Biochem. Cytol.* **4**, 349.
Rouget, C. (1862). *Compt. rend.* **55**, 548.
Rouget, C. (1864). *Compt. rend.* **59**, 851.
Savay, G., and Csillik, B. (1956). *Acta Morphol. Acad. Sci. Hung.* **6**, 289.
Sawyer, C. H., Davenport, C., and Alexander, L. M. (1950). *Anat. Record* **106**, 287
Snell, R. S., and MacIntyre, N. (1955). *Nature* **176**, 884.
Snell, R. S., and MacIntyre, N. (1956). *Brit. J. Exptl. Pathol.* **37**, 44.
Stohr, P. (1928). *In* "Handbuch der mikroskopischen Anatomie des Menschen" (W. von Mollendorff, ed.), Vol. 4, Pt. 1. Springer, Berlin.
Taxi, J (1957). *Compt. rend.* **245**, 564.
Taxi, J. (1958) *Compt. rend* **246**, 1922.
Tello, J. F. (1905). *Trabajos Lab Invest. Biol. Madrid* **4**, 105.
Tello, J. F. (1907). *Trabajos Lab. Invest. Biol. Madrid* **5**, 117.
Tello, J F. (1917). *Trabajos Lab. Invest. Biol. Madrid* **15**, 101.
Tello, J. F. (1944) *Trabajos Inst Cajal invest biol. Madrid* **36**, 1.
Tiegs, O. W. (1932). *J Anat.* **66**, 300.
Tiegs, O. W. (1953). *Physiol. Revs* **33**, 90.
Tower, S. S. (1931) *J. Comp. Neurol* **53**, 177
Valentin, G. (1836) *Nova Acta Phys. -med Acad. Caesareae Leopoldino-carolinae* **18**, 51.
Wagner, R. (1847) "Neue Untersuchungen uber den Bau und die Endigungen der Nerven." Leipzig (cited by Ranvier, 1878).
Weiss, P. (1941). *Growth* **5**, Suppl., 163
Woolf, A. L., and Till, K (1955) *Proc. Roy Soc. Med.* **48**, 189.
Zacks, S. I., and Welsh, J. H. (1951) *Am J. Physiol.* **165**, 620.

CHAPTER XI

Muscle Spindles and other Muscle Receptors

SYBIL COOPER

I. INTRODUCTION

The presence of sensory nerve endings in striated muscle is widespread throughout the animal kingdom, though the endings may be easier to identify in some species than in others. The most widely studied of these endings is the muscle spindle, whose history goes back just about one hundred years. Little groups of small diameter muscle fibers were seen in vertebrate muscles about 1850; Kolliker (1862), using the frog, and Kuhne (1863), using the mammal, showed that these groups were supplied by at least one large nerve fiber, often larger than the fibers to the motor end-plates. These groups of muscle fibers formed small entities, being partly encapsulated. Some people thought them to be pathological or developing muscle fibers, but their widespread occurrence suggested that they were normal structures. The possibility that the large nerve fibers supplied sensory endings in muscle was also discussed. As techniques for staining nerves were developed, similar large nerve fibers ending in spindle shaped organs were found in the muscles of reptiles and birds. Golgi (1880) described other encapsulated nerve endings at the musculotendinous junctions. There is also mention at this time of occasional Pacinian corpuscles and other smaller encapsulated endings near the tendons of some muscles.

Much histological work was carried out on the muscle spindle, but the clearest accounts of the nerve supply were first given by Cajal (1888, 1897) for the frog and by Ruffini (1892b, 1893, 1898) for the mammal (cat). The sensory nature of the spindles and tendon organs

Kuhne, W. (1887). *Z. Biol.* **23**, 1.
Kuhne, W. (1892). *Verhandl. d. nat. -med. Ver. Heidelberg* **4**, 1.
Kulchitsky, N. (1924). *J. Anat.* **59**, 1.
Kupfer, C. (1951). *J. Cellular Comp. Physiol.* **38**, 469.
Lawrentjew, B. J. (1928). *Z. mikroskop. anat. Forsch.* **13**, 388.
London, E. S., and Pesker, D. J. (1906). *Arch. mikroskop. Anat. u. Entwicklungsgesch.* **67**, 303.
MacManus, J. F. A. (1948). *Stain Technol.* **23**, 99.
Marnay, A., and Nachmansohn, D. (1937). *Compt. rend. soc. biol.* **125**, 41.
Naville, A. (1922). *Arch. biol. (Liège)* **32**, 37.
Noel, R. (1925). *Bull. histol. appl. et tech. microscop.* **2**, 124.
Noel, R. (1927). *Bull. histol. appl. et tech. microscop.* **4**, 382.
Noel, R. (1950). *Biol. méd. (Paris)* **39**, 273.
Palade, G. E., and Palay, S. L. (1954). *Anat. Record* **118**, 335.
Palay, S. L. (1956). *J. Biophys. Biochem. Cytol.* **2**, *Suppl.*, 192.
Palay, S. L. (1958). personal communication.
Quatrefages, A. de (1843). *Ann. sci. nat. Zool.* [2] **19**, 299.
Ranvier, L. (1878). "Leçons sur l'Histologie du Système nerveux." Savy, Paris.
Ranvier, L. (1888). "Traité technique d'histologie," 2nd ed. Savy, Paris.
Reger, J. F. (1954). *Anat. Record* **118**, 344.
Reger, J. F. (1955). *Anat. Record* **122**, 1.
Reger, J. F. (1957). *Exptl. Cell Research* **12**, 662.
Renaut, J. (1899). "Traité d'histologie pratique," Vol. 2, Pt 2, p. 972. Rueff, Paris.
Retzius, G. (1892). *Biol. Untersuch. [N. F.]* **3**, 41.
Rio-Hortega, P. del (1925). *Compt. rend. soc. biol.* **92**, Suppl., 3.
Robertson, J. D. (1954). *Anat. Record* **118**, 346.
Robertson, J. D. (1956). *J. Biophys. Biochem. Cytol.* **2**, 369.
Robertson, J. D. (1957). *J. Physiol. (London)* **40**, 58P.
Robertson, J. D. (1958). *J. Biophys. Biochem. Cytol.* **4**, 349.
Rouget, C. (1862). *Compt. rend.* **55**, 548.
Rouget, C. (1864). *Compt. rend.* **59**, 851.
Savay, G., and Csillik, B. (1956). *Acta Morphol. Acad. Sci. Hung.* **6**, 289.
Sawyer. C. H., Davenport, C., and Alexander, L. M. (1950). *Anat. Record* **106**, 287
Snell, R. S., and MacIntyre, N. (1955). *Nature* **176**, 884.
Snell, R. S., and MacIntyre, N. (1956). *Brit. J. Exptl. Pathol.* **37**, 44.
Stohr, P. (1928). *In* "Handbuch der mikroskopischen Anatomie des Menschen" (W. von Mollendorff, ed.), Vol. 4, Pt. 1. Springer, Berlin.
Taxi, J (1957). *Compt. rend.* **245**, 564.
Taxi, J. (1958). *Compt. rend.* **246**, 1922.
Tello, J. F. (1905). *Trabajos Lab. Invest. Biol. Madrid* **4**, 105.
Tello, J. F. (1907). *Trabajos Lab. Invest. Biol. Madrid* **5**, 117.
Tello, J. F (1917) *Trabajos Lab. Invest. Biol Madrid* **15**, 101.
Tello, J. F. (1944). *Trabajos Inst Cajal invest. biol. Madrid* **36**, 1.
Tiegs, O. W. (1932) *J Anat.* **66**, 300
Tiegs, O. W (1953). *Physiol. Revs* **33**, 90.
Tower, S. S (1931). *J Comp. Neurol.* **53**, 177.
Valentin, G. (1836). *Nova Acta Phys. -med Acad. Caesareae Leopoldino-carolinae* **18**, 51.
Wagner, R (1847). "Neue Untersuchungen uber den Bau und die Endigungen der Nerven." Leipzig (cited by Ranvier, 1878).
Weiss, P (1941). *Growth* **5**, Suppl., 163.
Woolf, A. L., and Till, K. (1955). *Proc. Roy Soc. Med.* **48**, 189.
Zacks, S. I., and Welsh, J H. (1951). *Am. J Physiol.* **165**, 620.

not then be confined to single groups of muscles, for important points may thereby be missed. There is much also to be learnt from histological and electrical studies of the muscles of the lower animals whose receptors are often simpler than those of mammals, though with similar underlying principles. The muscle fibers of mammalian muscle spindles also have features in common with the ordinary extrafusal muscle fibers of some of the cranial muscles of mammals and with the muscle fibers of the lower animals.

II. HISTOLOGY OF MUSCLE RECEPTORS

A. Mammalian Muscle Receptors

1. *Muscle spindles*

A vertebrate muscle spindle consists essentially of one or several muscle fibers of small diameter with some or all of their length enclosed in a connective tissue capsule. Inside the capsule are found the endings of one or more sensory nerves on these so-called intrafusal muscle fibers. Motor endings are also found on these muscle fibers both inside and outside the capsule. The sensory endings are usually supplied by large and the motor endings by smaller myelinated nerve fibers.

In true longitudinal sections of a mammalian muscle that has been kept straight during fixation, muscle spindles can be recognized as elegant spindle shaped structures often several millimeters long but only a few tenths of a millimeter broad at their widest part. The "spindle" gets its name from the long connective tissue laminated capsule; this contains the group of slender muscle fibers which receive a complicated nerve supply. In describing these organs I shall sometimes draw on personal observations to amplify the accounts of earlier workers.

The average number of intrafusal muscle fibers is fairly typical for each mammal, though the variation from the mean may be considerable. The number is said to be 4 in the rabbit, 6 in the cat, and about 10 in man and in the primates. In the latter group, the number may vary from 2 to 16. The details of the spindles containing many muscle fibers are hard to make out, especially if the organs have shortened in fixation. It was formerly stated that the little intrafusal fibers were red in nature; P. Kruger (1952) maintains that the intra- and extrafusal fibers have the same structure, i.e. that they would both be red in a red muscle or white in a white muscle. Since many muscles are mixed,

was reported rather briefly by Onanoff in 1890 and independently and very fully by Sherrington in 1894. Both authors cut dorsal spinal roots in mammals and found that the large nerve fibers in the muscle nerves and their endings degenerated completely. Sherrington at the same time gave a very clear description of the general structure of a muscle spindle.

Little new work on these organs was done for about 40 years. Sherrington frequently wrote about their sensory function, and was convinced that they played an important part in muscle tone and in the myotatic reflexes that he studied so profitably. The issue in 1920–30 was somewhat obscured by the general trend to associate tone with a possible sympathetic supply to muscle fibers. New advances came through electrophysiological methods, more especially from the technique, introduced by Adrian (1926; Adrian and Zotterman 1926) and developed by Matthews, of recording from single nervous units. Later realization of the importance of the motor supply to the muscle fibers of muscle spindles has brought a fuller understanding of tone and normal movement and is beginning to throw light on some of the neuromuscular diseases.

Many useful reviews on the sensory endings in muscle and their function have appeared, notably by Regaud and Favre (1904), Hinsey (1934), Tiegs (1953), and Granit (1955). A very full account of the structure of the mammalian muscle spindle (rabbit) is given by Barker (1948). These works make it possible to be brief about many important investigations and an attempt will be made here to bring together a number of perhaps less well known or new facts that may have some bearing on the whole story.

In studying muscle receptors, especially muscle spindles, it should not be overlooked that the muscles themselves may vary both in structure and in function. Some muscles are described as "quick", phasic, and white and are concerned with rapid movements; others are "slow", tonic, and red in nature and are much concerned with posture; often these characteristics are mixed in one muscle. Most vertebrate striated muscles bring about movements of the bony skeleton, but many of the cranial muscles and a few body muscles may be partially or wholly independent of bone. Such differences are dealt with elsewhere (see Volume I, Chapter II), but I wish to emphasize here that there may be differences in the structure and distribution of the receptors in these various kinds of muscle; histological or physiological work should

drawing information from as many methods as possible. Intravital methylene blue, gold chloride, and the silver techniques are used for the nerves and their endings. Myelin stains show the nerve sheaths only. The cholinesterase methods are now being used to give information about motor endings and new knowledge is being obtained by the use of the electron microscope. The methylene blue and the gold chloride methods have the merit that whole spindles may be investigated, though it does not follow that whole spindles are uniformly stained. For silver work, sections are used; if a whole spindle is to be studied, then serial sections are needed, from which the final picture must be built up. In longitudinal sections, this means the study of many fields of view (Barker, 1948); with transverse sections, the study of hundreds of sections may be necessary (Cooper and Daniel (1956) and in preparation). The intricate mixture of sensory and motor endings on the spindle muscle fibers can only be unravelled by cutting the motor or sensory roots; this is being undertaken by Boyd (1959).

It is clear, from a study of the nerves to spindles, that there are at least three types of muscle spindle, which Ruffini (1898) classified as simple, intermediate, and complex, depending on the number of sensory endings in each type. All the spindles have a primary ending at the equator, known also as the annulo-spiral or nuclear bag ending. In the simple spindles, this is the only sensory ending. In the intermediate spindles, there is one other recognized sensory ending, and in the complex spindles two or perhaps more of these other endings, which are known as secondary, flower-spray, or myotube endings. All the spindles have small, typical, motor end-plates on their larger muscle fibers and there are also more diffuse motor endings in the intermediate and complex spindles. The distribution of these three types of spindle in individual muscles will be discussed later. Very fine nerve fibers are found in the capsules and on the blood vessels of spindles.

a. Simple Spindles. Simple mammalian spindles (Fig. 1*a*) are comparatively short, they usually contain less than the average number of intrafusal muscle fibers, which all have well marked nuclear bags at their midregion; these fibers often pass out of the capsule at the poles. A large nerve fiber (12 to 18 μ in the cat) enters the capsule close to the equator, its internodal lengths shorten, and the fiber thickens before dividing up to send a branch to the region of each nuclear bag; at the bag the myelin sheath is lost and the ending proper starts. Each branch of this primary ending takes the form of a regular spiral

there is thus the possibility that some of their spindles may contain both types of muscle fiber; a point to which I shall refer later. The intrafusal muscle fibers run from one pole of the capsule to the other, but on closer examination or in serial transverse sections it is seen that the larger fibers pass beyond the capsule at one or both poles and end as fine threads on the perimysium of extrafusal muscle fibers (Fig. 4). No branching of intrafusal muscle fibers is seen, but the decrease in the number of these fibers when some leave the capsule at the poles may have suggested branching. The capsule itself is either attached to the perimysium, or to fascial septae, or fine connective tissue threads pass on in the intramuscular spaces to join a slip of tendon. The capsule is unbroken, it wraps closely round the issuing muscle fibers at the poles and round entering blood vessels; it is continuous with the sheaths of Henle of the incoming nerve fibers. Most of the little muscle fibers thus lie in an intracapsular or periaxial space; this is filled with fluid that may be connected with the lymphatic system (Sherrington, 1894). At the equator of the spindle, the intrafusal muscle fibers are modified. The larger may contain a collection of clear nuclei (Fig. 5) forming a nuclear bag which causes a swelling in the muscle fiber and thinning, though not abolition, of the overlying striated tissue (Cooper and Daniel, 1956). At each end of the bag, there may be a few single central nuclei with a thicker layer of striated tissue round them. Smaller intrafusal muscle fibers may not show these marked nuclear bags at the equator but instead they have a myotube region consisting of a row of elongated muscle nuclei lying along the center of the fiber; the striations in the muscle tissue overlying these nuclei are unmistakable, but when these myotubes run between the nuclear bags of adjacent muscle fibers, their diameter in this region may be small in fixed sections. It is these smaller fibers that end at the poles (Boyd, 1956). Some or perhaps all the intrafusal fibers with nuclear bags are the ones that pass out of the capsules. All muscle spindles have a good blood supply inside the capsule.

This description of a muscle spindle is based upon histological material, in single or serial sections, in which the nerve fibers are not stained. It is incomplete, and may give erroneous information when used on clinical material. It does, however, form the background upon which to apply the results of all the more difficult nerve staining techniques. These latter methods each give their individual picture and our final assessment of the spindles must be a composite picture,

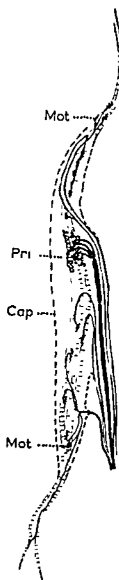
or of several rings attached to a central stem; it is closely applied to the surface of the attenuated striated substance of the muscle fiber lying over the clear nuclei of the "bag". Finally it breaks up into delicate sprays on the muscle. The whole ending may extend for 150 to 300 μ along the muscle fibers in a cat's muscle spindle (Fig. 1 and 3*a*). Merrillees (1957), from electron microscope studies, reports that the ending deeply indents the sarcolemma and the underlying thin layer of poorly striated myofibrils. The primary ending lies in the widest part of the spindle, and the capsule and its fluid content may thus form an insulating cushion protecting the ending from extraneous activity in the muscle. In addition to this primary sensory ending, these simple spindles have small motor end-plates on the muscle fibers; there are one or more plates on either half of each spindle muscle fiber. They are innervated by 3 to 8 μ nerve fibers, some of which can often be seen to divide to supply several endings.

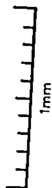
b. Intermediate Spindles. In the intermediate spindles (Fig. 1*b*), not only is there an extra sensory ending in addition to the primary ending, but between this ending and the pole there may be diffuse fine endings which are supplied by small myelinated nerve fibers and which are probably motor in function. Small motor end-plates are found on the intrafusal muscle fibers at both poles. The primary ending follows the same pattern as in the simple spindle and it always receives the largest nerve fiber to enter the spindle. It is flanked on one side by a somewhat similar though usually less regular ending. The single nerve fiber to this secondary ending is a little smaller (8–12 μ) than that to the primary ending. The final branches may each consist of an unmyelin-

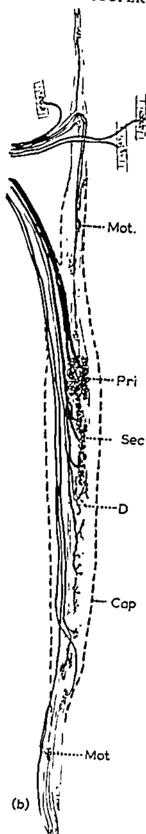
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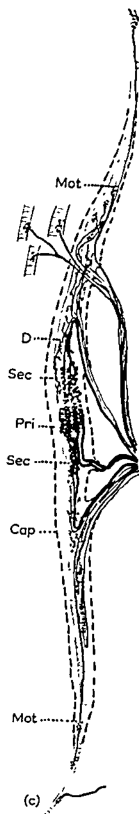
FIG. 1. Drawings of cat's muscle spindles in teased gold chloride preparations. Structures projected and outlined, further details filled in from microscope studies. *a.* Extensor digitorum longus. *Simple* spindle with primary ending, Pri; and motor end-plates, Mot. *b.* Tibialis anticus. *Intermediate* spindle with primary ending, Pri, and one secondary ending, Sec. Both poles have motor end-plates, Mot; but between the end-plates of the lower pole and the secondary ending there is a diffuse mass of endings, D, supplied by small nerve fibers. *c.* Soleus. *Complex* spindle with

nerve fibers. The scale applies to *a*, *b* and *c*. Note the greater length of the capsule when there is a secondary ending. All the spindles have some of their intrafusal muscle fibers running beyond the capsules, Cap. and they sometimes have small endplates on them here.


Mot
Pri
Cap
Mot
(a)


1mm


Mot.
Pri
Sec
D
Cap
Mot
(b)


Mot
D
Sec
Pri
Sec
Cap
Mot
(c)

In earlier degeneration experiments, after cutting spinal roots, the only distinction made in the spindle nerve supply was between sensory endings near the equator and motor endings at the poles; more evidence is forthcoming (Boyd, 1959) that should distinguish between the secondary endings and diffuse supposedly motor endings which may lie near them. The primary and secondary endings lie close together (in the equatorial region of the spindle), while the motor endings may extend from the small motor end-plates outside the capsule to the diffuse endings near or among the sensory endings. This would explain the great extent of motor endings seen by Coërs (1954) and Coërs and Durand (1956) in their cholinesterase studies of human spindles, which led them to suggest that the secondary endings were really motor endings. A scrutiny of the endings in a well stained cholinesterase longitudinal section of muscle often shows small discrete motor end-plates at the poles and a blurred pattern nearer the equator. The latter may well be due to diffuse motor endings intermingled with parts of the secondary endings. Physiologists postulate numerous motor endings on a single intrafusal muscle fiber which could set up local nonpropagated contractions. The diffuse endings might fulfil this function. There are only small motor end-plates at each end of the larger muscle fiber and this arrangement is much more suggestive of a twitch mechanism. The larger size of some of these polar motor nerve fibers may place them in the alpha category.

2. *Tendon Organs and Other Endings*

Tendon organs are found at the musculotendinous junctions, both at the ends of a muscle and at the tendinous aponeuroses found in so many muscles well away from the tendons of origin and insertion. Each organ is supplied by a single large myelinated nerve fiber (12—18 μ) which can often be traced for a long distance. This fiber divides many times as it reaches the ending, each branch terminating as a spray of fine endings that lie between the strands of tendon close to the ends of a group of muscle fibers, or actually at the musculotendinous junctions (Fig. 2 and 7). The whole organ usually has a thin capsule and any extension of the tendon brought about by passive stretch or active contraction of the muscle must cause pressure on the terminal sprays of the nerve ending. The large nerve fiber is often accompanied by a small one going to vessels. Pacinian and other smaller corpuscles enclosing nerve endings are sometimes found

ated stem with short twigs that clasp the muscle fiber and end as small sprays (Fig. 3*a* and *b*); in some animals, the final branches take a spiral form (Barker, 1948, rabbit). These endings, as well as the primary endings, start with a Schwann sheath; the final branches may finis as naked axons rich in mitochondria. The muscle fiber beneath this secondary ending is always striated and it contains a row of centrally placed elongated single nuclei. In these intermediate spindles, not all the muscle fibers have nuclear bags, for some have only myotubes at the equator (Cooper and Daniel, 1956, man; Boyd, 1958a, cat). The branches of the secondary ending are thickest on those muscle fibers that have no nuclear bags but is is possible that both primary and secondary endings do lie to some extent on all the intrafusal muscle fibers (Boyd, 1958a). The medium sized nerve fiber to this secondary ending is often accompanied by other small myelinated nerve fibers which spread over some of the intrafusal muscle fibers to end as diffuse grapelike clusters or as elongated atypical end plates (Fig. 3*b* and *c*). These endings often appear to lie close to or even among the branches of the secondary ending on the muscle fibers with no nuclear bags; they are always inside the capsule and differ from the small typical motor end-plates, which are again seen on the larger intrafusal muscle fibers at both poles, both inside and outside the capsule. Ruffini gave drawings of some of these varied endings in 1898. The diffuse endings get their nerve supply from the spindle nerve trunk, while the small motor end-plates may get theirs from trunks which supply the motor end-plates of the neighboring extrafusal muscle fibers. When there are two types of muscle fiber in these spindles, then the ones with the nuclear bags and small motor end-plates are probably phasic while those with myotubes and diffuse endings may be tonic in function.

Complex Spindles. The third or complex type of spindle (Fig. 1*c*) always has at least two secondary sensory endings. These may lie on either side of the primary ending or they may both be on one side of it. Their medium sized nerve fibers come from the same trunk as the fiber to the primary ending and can often be followed individually for some distance. There are always several fine nerve fibers which enter the spindle with the fibers to the secondary endings and run to elaborate fine endings of the diffuse grape type. Again, there are small motor end-plates on the larger intrafusal muscle fibers at both poles. These complex spindles are always the longest to be found in a muscle.

In earlier degeneration experiments, after cutting spinal roots, the only distinction made in the spindle nerve supply was between sensory endings near the equator and motor endings at the poles; more evidence is forthcoming (Boyd, 1959) that should distinguish between the secondary endings and diffuse supposedly motor endings which may lie near them. The primary and secondary endings lie close together (in the equatorial region of the spindle), while the motor endings may extend from the small motor end-plates outside the capsule to the diffuse endings near or among the sensory endings. This would explain the great extent of motor endings seen by Coers (1954) and Coërs and Durand (1956) in their cholinesterase studies of human spindles, which led them to suggest that the secondary endings were really motor endings. A scrutiny of the endings in a well stained cholinesterase longitudinal section of muscle often shows small discrete motor end-plates at the poles and a blurred pattern nearer the equator. The latter may well be due to diffuse motor endings intermingled with parts of the secondary endings. Physiologists postulate numerous motor endings on a single intrafusal muscle fiber which could set up local nonpropagated contractions. The diffuse endings might fulfil this function. There are only small motor end-plates at each end of the larger muscle fiber and this arrangement is much more suggestive of a twitch mechanism. The larger size of some of these polar motor nerve fibers may place them in the alpha category.

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a
b
c
d
100μ

near the tendons of muscles; they are particularly numerous in some of the small muscles of the hand and foot. These small sensory corpuscles usually lie alongside tendon organs (Fig. 2), but with a separate fair-sized nerve fiber (Ruffini, 1892a). They may be in little groups sharing a common nerve fiber (Fig. 8). Recently, Stilwell (1957a, b, c) has described the innervation of tendons in mammals. He finds that

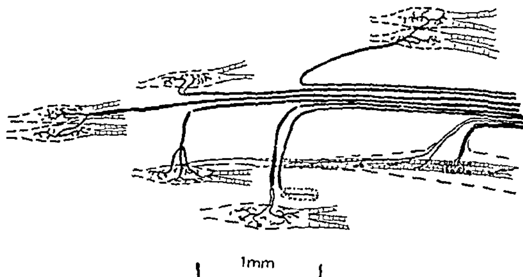


FIG. 2. Drawing of a small piece of the distal tendon of the cat's sartorius from a teased gold chloride preparation. There are five encapsulated tendon organs; two of these are double and the two parts share a nerve fiber, otherwise they each have a separate large nerve fiber. Half a simple muscle spindle is seen on the right; its muscle fibers pass beyond the capsule and finally end as tendinous threads attached to one side of a tendon organ; the nerves to the primary ending of the spindle and to this tendon organ are quite separate. Close to the lowest tendon organ is seen a small sensory corpuscle, again with a distinct nerve fiber

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FIG. 3. Untouched photomicrographs of four adjacent portions of an intermediate muscle spindle from the cat's soleus. Teased gold chloride preparation. In *a* is seen

hand half of *b*. The medium sized nerve fiber is accompanied by a small nerve fiber which divides and runs to the right edge of *b*. Three small nerve fibers are seen in the lower part of *a* and *b* and on the left edge of *c*. In *c*, one of these small fibers turns up and probably supplies some of the diffuse endings which stretch from the middle of *b*

the motor end-plates belonging to the other pole of the spindle can be seen.

In most muscles, the spindles are distributed throughout the belly of the muscle with their greatest concentration near the region of nerve entry. In studying serial transverse sections of parallel fibered muscles, the impression is received that if the whole muscle were projected on to one section, a part of that section would be very richly supplied with spindles. In other words, there is a core of muscle with numerous spindles and often successive spindles lie between the same muscle bundles in different transverse levels of the muscle (Fig. 4). Such spindles may be separate or often they overlap slightly and the ends of the intrafusal muscle fibers are dovetailed together. An extreme case of this is found in the tenuissimus of the cat where the 10 or more spindles are found in one long chain through a large part of the muscle (Boyd, 1956), the spindles again being dovetailed or slightly separated. It is not uncommon in various muscles for the distal member of such a spindle sequence to have its distal pole attached to the side of a tendon organ. There may be several other tendon organs near and sometimes one or two small sensory corpuscles; they all have discrete nerve fibers, which often run together in the same nerve trunk (Fig. 2). Some of these spindles in a spindle sequence appear to have a close relation with some of the neighboring extrafusal muscle fibers, for the latter receive their motor endings from the spindle nerve trunk and are partly enclosed in a lateral extension of the spindle capsule (Fig. 5). The musculotendinous junctions of these extrafusal fibers may be the site of one of the tendon endings. The group suggests a highly specialized unit that may be of physiological importance (personal observation).

4. *Cranial Muscles*

The somatic limb muscles and most of the trunk muscles have been developed in all mammals to move portions of the skeleton in relation to the joints, or to keep those joints fixed. They all contain sensory receptors which vary in number and structure, depending on the delicacy of the movements involved or on the part played by the muscles in maintaining posture. In addition, each of these muscles has a nerve whose fibers have known pathways to and from the spinal cord through the dorsal and ventral roots, the cells of origin of the receptors being situated in the dorsal root ganglia. The cranial muscles are developed for specific purposes in different mammals. The movement of the bony skeleton is less frequently the object of contraction and the pathways of

muscles shows that soleus has many long spindles, tibialis has numerous short spindles, while gastrocnemius has spindles of intermediate length. My own observations suggest that the long complex spindles are particularly numerous in the tonic muscles and the short simple spindles are more often found in the flexors which tend to be phasic in function. Voss (1937) reported many short spindles in the human lumbricals. Freimann (1954) stated that there were many big spindles in the deeper part of the human masseter; that part of the muscle, together with the spindle-rich medial pterygoid (Table I), may have a marked tonic function in maintaining the posture of the lower jaw. Temporalis had many small spindles; these may bear some relation to its function. The question of tonic and phasic fibers in a muscle is far from simple since many muscles are mixed (cf. Gordon and Phillips, 1953) and there are big variations in the same muscles of different species. The figures given by Danzinger (1936) for the relative numbers of muscle fibers of two differing structures in numerous mammalian muscles is of interest in this respect.

Other cat muscles having high spindle counts for the size of the muscle are the intercostals, 60 to 100 in each space (Huber, 1902); the fifth interosseous, 19 (Hines and Tower, 1928); and tenuissimus, 10 to 14 (Boyd, 1956). A study of specimen slides taken at 4 mm. intervals throughout the length of different muscles points to a high relative number of spindles in the muscles at the base of the cat's tail and also in the small muscles of the neck in man, especially in the superior oblique capitis (personal observation). These muscles are undoubtedly important both in initiating fine movement or in maintaining posture and many spindles might be expected in them (cf. Voss, 1958).

It would be of great interest to have some exact knowledge of the number and distribution of tendon organs in different muscles, but up to the present any available information is based on counts of large sensory nerve fibers to a muscle and on the assumption that the primary ending of each spindle is connected to one of these fibers; any large fibers in excess of the total spindle number are assumed to come from tendon organs (Hunt, 1954). This may give a useful guide, but it is indirect and may be erroneous; more figures are necessary for both nerve counts and spindle counts to explain certain discrepancies. Some muscles have small nerve trunks running apart from the main muscle nerve; these may get overlooked in nerve counts, though they often contain a high proportion of sensory fibers.

the sensory nerve fibers are still in some cases a matter for speculation. It is better then to consider these muscles separately from the limb muscles, though many of the lessons to be learned from a study of their receptors can be applied directly to the limb muscle receptors.

a. Jaw Muscles. The muscles which most closely resemble the limb muscles in function are the jaw muscles developed in all mammals to move the lower jaw and to maintain its posture. These muscles also resemble the limb muscles in the structure of their extrafusal muscle fibers and their muscle spindles. Table I gives Freimann's figures for the spindle numbers in the jaw muscles in man; it is puzzling that the external pterygoid is without spindles. I found that this was also the case in the goat and cat, yet all these muscles are supplied by the fifth cranial nerve. Among other muscles supplied by the fifth nerve, Voss (1956c) found a few spindles in the anterior belly of the digastric. The cells of origin of the sensory nerves from the muscle spindles in the jaw muscles lie in the mesencephalic nucleus of the fifth nerve (Corbin and Harrison, 1940; Cooper *et al.*, 1953), i.e. they lie inside the brainstem and, as far as we know, both motor and sensory pathways to the brain are in the motor root of the fifth nerve. The jaw jerk functions through a monosynaptic reflex arc (Szentágothai, 1948).

FIG. 4. Human lumbrical from the hand. Transverse section, 15μ, Holmes' silver method. In the intramuscular space, the lower arrow points to three tiny muscle fibers known, from a study of serial sections, to be the ends of spindle muscle fibers which have left the capsule and are about to finish on the perimysium of extrafusal

FIG. 5. Human lumbrical from the hand. Transverse section, 7μ, Holmes' silver method. The equator of a muscle spindle is seen; there are three large nuclear bags whose clear nuclei are surrounded by thin muscle tissue and parts of the poorly stained primary ending. The ending on the right hand "bag" has a Schwann nucleus on its upper edge. There are six small intrafusal muscle fibers to the right of the bags, some have central nuclei. The capsule of this spindle extends to form a cuff round a group of neighboring extrafusal muscle fibers.

FIG. 6. Human extrinsic eye muscle. Transverse section, 15μ, hematoxylin and

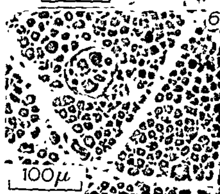
FIG 8. Three sensory corpuscles from the edge of a baboon's thenar muscle. Teased gold chloride preparations, untouched photomicrograph. The three corpuscles are supplied by a single large nerve fiber.


5
100μ


500μ


8
7
250μ


4
50μ


6
100μ

the muscle spindles in the artiodactyls travel to the brainstem in nerves which usually leave the muscles separately from the motor nerves and run to join the ophthalmic branch of the fifth nerve (Winckler, 1937; Whitteridge, 1955). The cells of origin are in the mesencephalic nucleus of the fifth nerve (Cooper *et al.*, 1953). Small modified tendon organs are numerous in eye muscles.

c. Facial Muscles. The facial muscles supplied by the seventh cranial nerve were said to contain no sensory receptors, but Bowden and Mahran (1956), working on the quadratus labii superioris of the rabbit, and Kadanoff (1956), working on human mimetic muscles, found in both cases a few unmistakable muscle spindles, as well as other endings that differed considerably from the known types of motor ending and are presumably sensory. Voss (1956c) reported 6 spindles in the human stylohyoid (Table I); he found no spindles in the posterior belly of the digastric.

d. Intrinsic Muscles of the Larynx. The instrinsic muscles of the larynx are another group of cranial muscles about which there has been speculation concerning their sensory supply. Sherrington (1897) reported that he was unable to find spindles here, but right up to the end of his life he was convinced that if a further search were made or electrophysiological investigations carried out, there would be evidence of the proprioceptors that he held must be present for the proper functioning of these delicate muscles. Goerttler (1950) shows a spindle in a human laryngeal muscle, and a few spindles have recently been reported in all the intrinsic laryngeal muscles of man by Lucas Keene (1957) and Paulsen (1958). Many of the extrafusal muscle fibers in these muscles are small in cross section, they have a rich nerve supply, and probably a search would reveal other atypical sensory endings on them. Andrew (1955) was unable to record sensory discharges in the external laryngeal nerve of the rat in response to stretching the cricothyroid muscle, but the sensory fibers from the muscle may not lie in this nerve.

e. Intrinsic Muscles of the Tongue. The intrinsic muscles of the tongue receive their motor nerve supply through the twelfth cranial nerve. Numerous muscle spindles have been found in these muscles in man and in the monkey (Cooper, 1953), but there are no spindles in the cat's intrinsic tongue muscles; nevertheless, low threshold discharges from stretch receptors have been recorded in the fibers of the twelfth nerve close to the tongue in these animals (Cooper, 1954). Again, the

b. Extrinsic Eye Muscles. In discussing the extrinsic eye muscles, their various uses must be remembered; they are primarily used to give the animal a stable field of view. The oblique muscles can usually rotate the eyes, but in some animals the recti move the eyes very little and it is movements of the neck that are closely linked with the position of the retinal images. In other animals and certainly in man, the eyes are very mobile and are constantly moved voluntarily as well as reflexly. Eye muscles in different animals show varied traits: they remind one of embryological muscle and also of limb intrafusal muscle fibers, their thin and thick muscle fibers recall the double muscle fiber system of some frog muscles (Kuffler, 1953), their nerve endings are more varied than those in the mammalian limb muscles and sometimes resemble endings found in the muscles of lower vertebrates. So far, muscle spindles have only been found in the eye muscles of man, of the higher primates, and of the artiodactyl branch of the ungulates (Cooper and Daniel, 1949), but stretch receptors are probably present in all eye muscles (Cooper and Fillenz, 1955). In the sheep, Cilimbaris (1910) reported up to 280 spindles in a single eye muscle, while we found about 120 in the inferior oblique of the goat (Cooper *et al.*, 1951). In man, there are about 50 spindles in the inferior rectus (Cooper and Daniel, 1949), a number that compares well with that for the first lumbrical, which is of similar weight. These spindles in the human eye muscles are best seen in cross section (Fig. 6), they have thin capsules and are found in certain parts of the muscle only, notably at the origin end and near the layer of thin muscle fibers at the circumference. They are more difficult to see in longitudinal section, probably because the capsules are short and confined to the region of the sensory endings, so that the greater part of the spindle muscle fibers lies outside the capsule, as in the spindles of the frog and other lower vertebrates. In man, the spindle muscle fibers have some central nuclei, but nuclear bags have not been seen. In the goat and sheep, there are well marked nuclear bags with primary nerve endings (Cilimbaris, 1910, sheep; Cooper *et al.*, 1955, man and goat). In human eye muscles, some of the myelinated nerve fibers take several turns round a muscle fiber before reaching a small ending on that fiber (Daniel, 1946). Similar myelinated nerve fiber spirals are sometimes seen on the intrafusal muscle fibers of human limb muscle spindles at some distance from the equator (Cooper and Daniel, 1956; Coers and Durand, 1956). The function of this type of ending is still in question. The impulses from

and vertebrates. He does not deal with the motor supply to muscle spindles, but the motor innervation of muscle in the lower animals and of the intrafusal muscle fibers in mammals do seem to me to have points in common.

1. *Invertebrates*

Receptors have been found in the muscles of arthropods, and accounts are available of crustacean receptors (Alexandrowicz, 1951, lobster; Kuffler, 1954, lobster and crayfish; Florey and Florey, 1955, crayfish) and insect receptors (Finlayson and Loewenstein, 1958; Slifer and Finlayson, 1956). The crustacean receptors consist of two types of specialized muscle fiber, each with its own sensory and motor supply. One fiber has coarse cross-striations, the other has finer cross-striations and more marked longitudinal striations. Both fibers have the muscular tissue modified under the dendrites of the large adjacent sensory nerve cells; Alexandrowicz states that in the lobster the striations are lost completely, but Florey and Florey are emphatic that there is definite muscle tissue in this region in the "slow" coarsely striated fiber of the crayfish and they assume that the region is just as contractile as the rest of the muscle fiber (cf. the muscle under the secondary ending of the mammalian muscle spindle). In the "fast" finely striated fiber, the central region is enlarged by an accumulation of connective tissue. The "slow" fibers have densely distributed motor endings from thin nerve fibers. The "fast" fibers have a large motor fiber which divides to supply both ends of the receptor.

2. *Vertebrates*

There is an interesting observation by Allen (1917) that eight or more muscle spindles are present in the cordis caudalis muscle of the agnathous vertebrate (*Polistotrema Bdellostoma*), a hagfish. He describes a laminated capsule and 3 intrafusal muscle fibers associated with a large nerve fiber and a spiral or annular nerve placque.

a. Fish. Fish have always presented a problem as regards muscle receptors. Giacomini (1898) and others were unable to find spindles in their muscles, though fine pencil shaped endings were reported (Poloumordwinoff, 1899). In 1937, Fessard and Sand, using electrical recording reported slowly adapting stretch receptors in the muscle of the cartilaginous ray and dogfish. Kirsche (1948) found endings in teleost muscle which he said had a structure related to the muscle spindle

stretch receptors are found in a typical form in some animals and are present in an atypical form in others. The impulses may finally reach the spinal cord by the ansa hypoglossus and the second cervical dorsal root.

Thus whenever a cranial muscle has been investigated by the special histological techniques for nerve endings or by electrical techniques, evidence of stretch receptors has usually been found. Interesting species differences and knowledge of the sensory pathways have also come to light. Little work has been done on the cranial muscles of lower vertebrates.

5. *Other Non-Joint-Moving Muscles.*

A few other non-joint-moving muscles in the body may be mentioned here. Typical spindles have been found in the striated esophageal muscles by Slawik (1942), in the cremaster muscle by Schinkele (1949), and in the diaphragm by Dogiel (1902) and by Winckler and Delaloye (1957), all in man. Andrew has electrical evidence of stretch receptors in the rat's esophageal muscle, while responses in the phrenic nerve to stretch of the diaphragm were recorded by Cardin (1944) in the rabbit. The sphincter muscles have not been fully investigated. Gerebtzoff and Grieten (1956) state that the anal sphincter of the rabbit and the urethral sphincter of the guinea pig are richly supplied with intrafusal fibers. Their criterion of such fibers is the presence of a series of motor endings on a single muscle fiber, not the presence of sensory endings or a capsule. These muscle fibers may indeed belong to spindles, or more probably they may be similar to the "small" muscle fibers of the lower vertebrates. Garry and Garven (1957) investigated the urethra of the cat and found spindles in the associated striated muscle.

B. Submammalian Muscle Receptors

The structure of the muscle receptors in the lower animals is often simpler than that of the complicated mammalian muscle spindles. There may be two separate spindle-like organs, each of which has some features of the mammalian organ. The motor endings of the receptor's muscle fibers, need to be studied in conjunction with the ending, to the ordinary muscle fibers, since both sets are often supplied by common nerve fibers. Hoyle (1957), in his recent book, gives a comparative account of motor innervation of muscle in both invertebrates

fibers. The spindle muscle fibers in the frog are often of great length and they may have a series of short encapsulated sensory regions (Cipollone, 1897; Vihvelin, 1932). Gray (1957) found one or sometimes two of these compound spindles in the extensor digitorum longus IV, each with up to five sensory regions and intervening regions receiving motor endings. In the toad pectoral muscle, which is sometimes called on for prolonged postural activity, Vihvelin reports 175 spindles per gram of muscle. Pezard and May (1937) counted the sensory endings in the sartorius of the frog and found 19, mainly at the tibial end of the muscle and along the internal border.

The electron microscope has recently made possible a more detailed study of a sensory ending in a frog's spindle [Robertson (1957) and see Gray (1959) Fig. 3b]. In serial transverse sections of an intrafusal fiber which has myofibrils in the attenuated sarcoplasm surrounding a central nucleus, the muscle surface is in ridges and grooves; on the former lie the unmyelinated strands of the nerve ending, some with Schwann cells, contacting the muscle fiber. The terminal portion of the ending contains many mitochondria and the Schwann sheath is lost.

d. Reptiles. The muscle spindles of snakes and lizards are mainly unifascicular, but they are of two types. One has a short capsule; there is a large sensory nerve fiber ending in a spiral round a modified part of the muscle fiber which is swollen, rich in nuclei, and poor in striations. Little motor end-plates are found at both ends of the muscle fiber outside the capsule; many of these small motor endings are ultraterminal endings from the motor end-plates of the ordinary muscle fibers. The other type of spindle has a long capsule, the sensory ending is much more spread out over muscle which retains its striations but has a row of central nuclei. This ending has been described by Cipollone (1897) as being similar to the flower spray or secondary endings of mammalian spindles. These reptile spindles receive several motor endings, but there is some confusion as to whether they are all of the motor end-plate type or whether some are the more diffuse grape endings. Tortoises have similar spindles, but they are multifascicular.

e. Birds. The sensory endings in bird muscles resemble much more closely those in mammalian muscles; there are complicated muscle spindles which are encapsulated, multifascicular, and richly supplied with nerve fibers. Bags of nuclei are very prominent, primary spiral endings are present, and probably there are secondary endings as well in some of the spindles (personal observation). Further work on bird

endings of the higher vertebrates. Barets (1952, and again in 1956) reported myoseptal pencil shaped endings in the dogfish which he believed to be the sensory endings investigated by Fessard.

b. Tailed Amphibians. In the muscles of the newt, *Triturus torosus*, Mather and Hines (1934) found three kinds of sensory ending. One was on musculotendinous junctions and was a basket-like structure. The others were clasplike endings found in the bellies of muscles, some on a single muscle fiber, others on groups of three small fibers. These muscle fibers were without capsules, but sometimes small motor endings were seen on them. This fact was confirmed by degeneration experiments. The pectoralis muscle contained both the single and the grouped muscle fibers with sensory endings, while many other muscles had one or other type only of these primitive muscle spindles.

c. Tailless Amphibians. The frog and toad have had their muscles subjected to many histological and physiological investigations. Cajal (1888, 1897), using the sternocutaneous muscle, shows a plurifascicular spindle with a short capsule. A single large nerve fiber enters the capsule and divides to end in long strands on the modified midregion of the muscle fibers; other nerve fibers go to typical frog brush motor endings on the spindle muscle fibers outside the capsule. Franqué (1891) gives a drawing of the midregion of a frog spindle; the capsule is short and there are two muscle fibers, each containing many lightly stained nuclei resembling those in the nuclear bags found under the primary endings of mammalian spindles; he states that the muscle striations are absent here. Recently, E. G. Gray (1957) made a detailed study of the extensor digitorum longus IV muscle of the frog. He describes the large sensory nerve fiber approaching the midregion of the spindle, usually from the side; it divides more than once, coils round the muscle fibers inside the short capsule, and ends in a "flower spray" ending. Under the endings, the nuclei, in one of Gray's photomicrographs, look like rows of myotube nuclei, rather than a collection of clear nuclei. The spindle muscle fibers have both typical frog motor end brushes and small grape endings, probably on separate fibers. Gray's account is based on the spindles of one small muscle; there are statements in the literature that there are two kinds of spindle in the frog (cf. Vihvelin, 1932) and this point has struck me on looking at spindles in various frog muscles stained with gold chloride. It would probably be worth examining spindles in a muscle with many "slow" fibers, such as the ileofibularis bundle, and in another like the sartorius, with no "slow"

either side of the bag. The myotube must also be stretched during passive stretch of the whole spindle, but the muscle there over the central nuclei is thicker and would presumably contract during motor activity in that part of the intrafusal fibers. The sensory endings in spindles and also in the tendon organs and Pacinian corpuscles respond to their adequate stimulus by initiating impulses which travel to the central nervous system. Histologically, we need more investigations at very high magnifications and also histochemical work to understand *the mechanism that fires off a sensory ending.* Katz (1950, a, b,) studied the sensory ending in a frog's muscle spindle electrically, by placing a small electrode close to the ending. J. A. B. Gray and his collaborators (1959) have done much work on the Pacinian corpuscles of the cat's mesentery, where they could place a microelectrode close to the nerve fiber as it entered the isolated corpuscle. A suitable stimulus to a sensory organ probably causes a deformation of the actual ending, leading to a depolarization of its lining membrane. If the membrane activity is great enough, then an impulse is initiated, possibly at the first node of Ranvier, and is propagated along the nerve. The end of the stimulus may result in a period of depression or hyperpolarization at the ending. The membrane surfaces of the sensory nerve endings in the spindle and in the tendon organ are relatively very large and they are capable of bringing about prolonged rhythmical activity in the sensory nerve fibers, of the order of about 5 to 100 impulses per second; the sensitive primary endings may discharge up to 500 impulses per second in response to a rapid stretch. With a small steady stretch, the endings may discharge for hours with very little change in rate. We know very little about the chemical changes that must take place at the spindle sensory endings; acetylcholine (Hunt, 1952) and succinylcholine (Granit *et al.,* 1953) (see Volume III, Chapter I) increase the sensory discharge but this may be due to increased activity of the spindle motor endings on the intrafusal fibers, since the effect is reduced by D-tubocurarine; no cholinesterase can be detected at the ending (Coers, 1954). In the Pacinian corpuscle, Hebb and Hill (1955) found pseudocholinesterase close to the nerve ending. This may also be the case in other sensory endings.

B. Discharges from Sensory Receptors

Most of our knowledge of the behavior of the muscle receptors comes from single receptor or single nerve fiber techniques. Matthews

muscles would probably be rewarding, for many birds have markedly phasic and tonic muscles which might show interesting differences in their spindles. Typical tendon endings are present and many Herbst corpuscles, rather similar to Pacinian corpuscles, lie close to the long tendons in the legs.

III. THE PHYSIOLOGY OF MUSCLE RECEPTORS

In discussing the physiological work on muscle receptors, there are certain points arising out of the histological work that are of particular interest. The end-plates on the spindle muscle fibers are undoubtedly motor and may cause twitches just as the motor endings do with ordinary muscle fibers. In many lower vertebrates, a second type of motor ending is seen in the main muscle, associated with slowly contracting muscle fibers. In some animals, these more diffuse grape endings are also seen on some of the spindle muscle fibers. There are now proofs that the mammalian muscle spindles have a diffuse second motor supply, which may be the mammalian counterpart of the "slow" system of the lower vertebrates (Boyd, 1958a, b). In some lower animals, two types of stretch receptor are seen, each with a different kind of muscle fiber and sensory ending and perhaps with *different types of motor ending. In the frog, it appears that there may be* two kinds of motor ending on separate spindle muscle fibers that share a sensory ending. In the mammal, there is a simple type of spindle with a primary sensory ending and motor end-plates, but there are also many spindles with two different sensory endings and two types of motor ending on the intrafusal muscle fibers; the exact relationship of these four types of ending needs further investigation, for it may have considerable bearing on the behavior of the muscle fibers and the sensory endings in the spindle. Since these complicated spindles also have two types of muscle fiber, we might expect to find such spindles in muscles with two types of ordinary muscle fiber; a particular bias towards numerous "slow" intrafusal muscle fibers, secondary endings, and diffuse motor endings would then be expected in the slower postural muscles, where indeed many complex spindles do occur.

A. The Initiation of Sensory Impulses

The histological evidence suggests that the thin layer of striated muscle lying over the nuclear bags may be stretched either by stretch of the whole spindle or by contraction of the intrafusal muscle fiber on

of his experiments, he was unable to measure conduction rates so as to have confirmatory evidence for the primary and secondary endings.

When the muscle nerve or the motor roots are cut, it is known, from a study of the motor fibers in the nerve histogram of a deafferented muscle (Eccles and Sherrington, 1930), that a big group of small motor nerve fibers has been cut as well as the large motor nerve fibers. Langley suggested in 1922 that such small fibers, seen by him in the anterior roots of cats, "may perhaps form the small nerve endings in muscle spindles."

The proof of this was not obtained until Leksell (1945) blocked the larger (8–15μ) nerve fibers to a muscle in the cat; then, on stimulating the small nerve fibers (3–8μ) or gamma efferents, as he named them, he was able to increase the discharges from the spindle endings with no observed increase of tension in the muscle. Kuffler and Hunt (1952) then carried out a series of experiments in which they stimulated a small fiber in a motor root and led from a sensory root fiber coming from the spindle supplied by the motor fiber. They showed that one motor fiber could affect several sensory endings, presumably in different spindles, and one spindle sensory ending could be affected by several small motor fibers. They suggested that the fusion frequency of the intrafusal muscle fibers was high, thus a rapid rate of stimulation to the small motor fibers might cause discrete contractile increments in the spindle muscle fibers, comparable with those found by Cooper and Eccles (1930) in the eye muscles. But they had also written of the small nerves causing local nonpropagated contractions similar to those found in the "slow" muscle fibers of frogs (Hunt and Kuffler, 1951 a). The first statement suggests a twitch mechanism and the second a slow tonic behavior; there is probably a place for both mechanisms.

The effects of small nerve stimulation are greatly modified by the tension on the muscle. In life, the small nerves may adjust the stretch on the spindle afferent endings during the varied activities of the intact muscle. The muscle thus has, in the spindle receptors, a means of signaling its own activity. Furthermore, these receptors are also controlled by a special motor supply to the small muscle fibers in the spindle. The tendon organs are unaffected by this gamma motor system.

The secondary endings of muscle spindles are supplied by nerve fibers of medium diameter; conduction studies by Merton (1953) confirmed that some discharges from stretch afferents in muscle reach

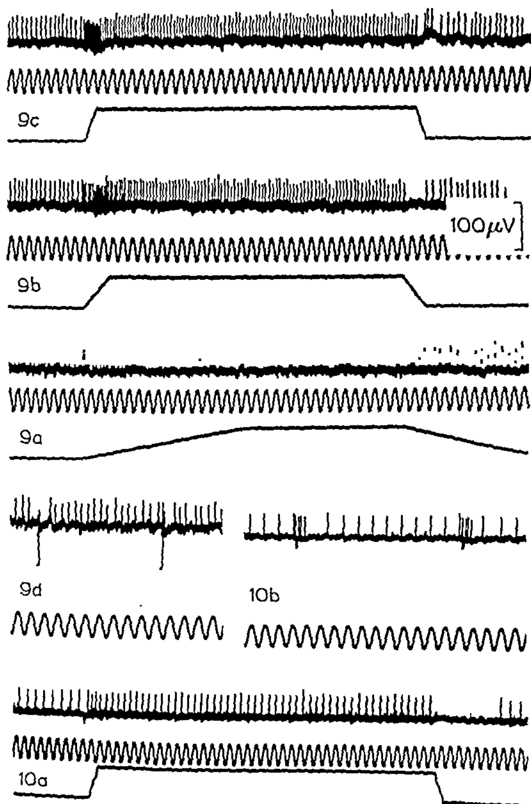
developed these techniques for muscles, using first a single spindle in a frog's muscle (1931a, b) and later cutting down the nerves from cat muscles till he was able to lead from a single afferent fiber (Matthews, 1933). In this mammalian material, he obtained responses from three types of ending, which he called A_1, A_2, and B, together with smaller slower impulses from so-called C endings, which he took to be fine pain endings in the fascia. The first three types of ending responded to stretch of the muscle.

The A_1 endings, which he ascribed to the secondary endings of spindles, had a low threshold. Their discharge, which sometimes reached some hundred impulses a second, varied with the rate and amplitude of stretch. They adapted slowly, but often stopped discharging for a brief period when the stretch was taken off. They never responded during active contraction of the muscle, but often started again during the period of relaxation. Their time relations were slower than those of the other two endings, suggesting that the discharges were travelling in smaller nerve fibers. The endings represented about 50% of those recorded from the hindlimb ankle extensors and flexors.

The A_2 endings, ascribed to the primary endings of the spindles, had a slightly higher threshold. They responded to stretch in the same way as the A_1 endings. They were silent during a submaximal or maximal twitch, but if the stimulus was made supramaximal and there was an adequate resting tension, giving a good resting discharge, then there was a discharge during the rising phase of the contraction. This was clearly seen in tetanus at a slow rate, for as the tension rose, the gaps between the stimulus artifacts filled up and there was a brisk sensory discharge during the plateau of the tetanus. The fast time relations of these sensory impulses suggested that they travelled in large nerve fibers.

The B endings, probably tendon endings, had a high threshold for stretch. They responded well to the degree of stretch, but less well to the rate of change of stretch. They always responded during active contraction and they had fast time relations. They amounted to 25% of all the endings responding to stretch.

Matthews suggested that during supramaximal stimulation of the muscle, the small fibers to the motor endings of the spindle muscle fibers were stimulated, causing contraction of the more heavily striated parts of these fibers and thus stretching the primary endings and reducing the stretch on the secondary endings. But, by the nature


9c
100 μV
9b
9a
9d
10b
10a

the dorsal roots later than the discharges travelling in the large sensory fibers. Hunt (1954), making a systematic study of the conduction rates of the fibers from stretch receptors in the cat's soleus and gastrocnemius, found ample evidence for discharges travelling in medium sized sensory fibers at about 50 m. per second compared with discharges travelling at 100 m. per second in the large fibers. He confirmed the high threshold of the tendon organs to stretch, but states that the spindle primary endings have a lower threshold than the secondary endings. This is the reverse of Matthew's findings.

Hunt and Kuffler (1951b) thought that the majority of the A group of receptors were primary endings. But Matthew's group A_1 may in fact have been a mixture of primary and secondary endings.

The responses of secondary endings to motor nerve stimulation must depend on how the intrafusal muscle fibers respond. In many muscle spindles, recent histological evidence now shows that there are motor fibers of different sizes supplying at least two kinds of motor ending; there are also large and small intrafusal muscle fibers. Matthews probably stimulated the larger motor fibers with his supramaximal shocks, but may not have affected the small motor fibers. Hunt and Kuffler selectively stimulated single small fibers in the ventral roots with much stronger shocks. These two kinds of stimulation might have different effects in the spindle, depending on which intrafusal muscle fibers contracted; also on whether one or both ends

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Fig. 9 Responses to stretching the superior oblique muscle of the goat's orbit, showing the discharges of a muscle spindle ending recorded in an afferent branch passing from the muscle to the fifth cranial nerve. The fourth cranial nerve was intact, so that the discharge is somewhat irregular and has a high resting rate. The stretches were applied at successively higher rates in records *a* to *c*, the same degree of stretch was reached in each case. In *d* are seen responses of the unit to a maximal shock to the fourth nerve, there is silence during the rising phase (10 msec.) of the twitch. Time 50 c./s.; stretch signal in *a*, *b* and *c*. Spikes retouched.

responses of the unit to a maximal shock to the fourth nerve; there is a burst of impulses during the rising phase of the twitch Time 50 c./s.; stretch signal in *a*. Spikes retouched.

muscle fiber contracted in response to several stimuli to the muscle nerve; again the sensory ending discharged. In 1958 Eyzaguirre was able to combine these fast and slow effects. Thus we have electrical evidence of two types of motor ending, one of the twitch and the other of the tonic type, on different spindle muscle fibers but affecting the same sensory ending. This links up well with E. G. Gray's histological evidence (1957).

Wiersma *et al.* (1953) recorded responses to stretch from the two muscle receptors in an invertebrate (crayfish). Kuffler (1954) showed in the crayfish and lobster that the receptor with the large finely striated muscle fiber gives large impulses and that this discharge is most affected by a changing rate of stretch. The fiber gives a twitch-like contraction if the motor nerve is stimulated and a high rate of stimulation is needed to produce a fused tetanus. The receptor of the small coarsely striated muscle fiber has a low threshold to stretch when the stretch organ is isolated; it is particularly responsive to the degree of stretch. On stimulation of the motor nerve, contractions can be seen but cannot be recorded in the muscle fiber and tetanus at low rates always gave a smooth contraction. The sensory discharges of both receptors were very sensitive to the effects of motor stimuli. Here is a primitive double stretch receptor, its two parts calling to mind the two types of spindle found in the muscle of many of the lower vertebrates, and it may be possible that the complex spindles of mammals behave in essentially the same way. The adjacent sensory cells of these crayfish receptors lend themselves to intracellular recording. Useful findings on the properties of sensory neurons have resulted, including knowledge of their behavior when inhibited, since the sensory cells have inhibitory fibers ending on them (Eyzaguirre and Kuffler, 1955; Kuffler and Eyzaguirre, 1955; Burgen and Kuffler, 1957).

The motor activity in a spindle greatly affects the discharge of the sensory endings which in turn influence the reflex behaviour of the muscle. Indeed P. B. C. Matthews and Rushworth (1957) have shown that by applying procaine to a muscle nerve in a decerebrate cat, and thus narcotizing the small nerve fibers before the large ones, it is possible to reduce the stretch reflex in the muscle even though the large alpha fibers usually associated with the reflex are still conducting. An increase of muscle tension is then required before the stretch reflex can be obtained again (Matthews, 1958).

of the fiber contracted, since all the fibers have a motor innervation at either pole. The physiological and histological work being carried out at the present time may give us a better understanding of how the discharges of the primary and secondary endings are modified and controlled by the activities of the different motor endings.

Results obtained from the physiological study of cranial muscles and their stretch receptors may throw further light on the activities of mammalian limb muscle spindles. Some of the cranial muscles may also prove to be the link between limb intrafusal muscle fibers in the mammal and the ordinary muscle fibers of some of the lower vertebrates. The work of Brown and Harvey (1938a, b) on neuromuscular conduction and on the effects of drugs on bird muscle makes interesting reading when compared with work on the mammalian extrinsic eye muscles and on the intrafusal fibers in limb muscles. The responses to stretch of eye muscle receptors (Figs. 9 and 10), recorded from single sensory nerve fibers, are similar to those recorded from the limb muscle receptors (Cooper *et al.*, 1951, 1953, 1955; Cooper and Fillenz, 1955). There are many muscle spindles in the artiodactyl eye muscles and the separate motor and sensory nerves to these muscles make them very useful for physiological work (Whitteridge, 1955). The primary endings were found to be very sensitive to any change in the rate of stretch when the spindle motor supply was intact (Cooper and Daniel, 1957). Whitteridge (1959) has very clear evidence of the effects of gamma fiber stimulation, in the goat's fourth cranial nerve, on the afferent discharges of spindle endings in the superior oblique muscle recorded in a branch of the fifth nerve.

Among the lower vertebrates most work has been done on the muscle spindles of the frog and toad. In 1949 Katz suggested from his work on the frog that there might be a double motor supply to the spindles, one rapid and the other slow, both supplies being closely related to the nerve supplies to the "fast" and "slow" muscle fibers in many frog muscles (Kuffler, 1953). Recently, microelectrodes have been inserted into some of the spindle muscle fibers in amphibians. Eyzaguirre (1957) using the extensor digitorum longus IV muscle of the toad, was able to record twitches in these fibers, and a sensory discharge was recorded simultaneously in the afferent fibers from the spindle. He got evidence also of slow muscle fibers belonging to the spindle but could not get an electrode into them. These slow fibers received motor endings from several small nerves. In lightly curarized muscle, the spindle

gamma activity. The main alpha activity is being dealt with in Volume II, Chapter II and I must confine myself here to the part played by the muscle receptors and their motor nerves.

There is good evidence for two sizes of motor nerve fiber to muscle spindles (Barker, 1948; Cooper and Daniel, 1956). It is the smaller fibers supplying the diffuse endings on the intrafusal fibers that probably form part of the gamma system. The larger nerve fibers can sometimes be seen dividing up to supply the little motor end-plates; we do not know whether their cells of origin fall within the alpha or the gamma category. Nor do we know how the motor supply of the simple spindles, with primary sensory endings only, links up with the gamma system. The recent work on phasic and tonic alpha motor nerves to a muscle (Granit *et al.*, 1957) and Pascoe's work on small alpha nerves (1958) may have bearings on the motor innervation of muscle spindles.

D. Functions of Muscle Receptors

When the discharges from the muscle receptors reach the central nervous system, their main function is to modify the activity of the alpha motoneurons. The most direct effect is brought about by the monosynaptic connection between the large nerve fibers from the primary endings of the spindles with the alpha motoneurons to the extrafusal muscle fibers in the same muscle, particularly to the fibers in that part of the muscle lying near those sensory endings. Under suitable conditions, the discharges from the primary endings can cause a stretch reflex in the muscle. A sharp stretch may bring about a reflex jerk; this happens in a few mseconds in either extensors (Jolly, 1911) or flexors (Asayama, 1916). A more gradual stretch may cause prolonged stretch reflexes in the extensors if the initial tone in the muscle is high. In the frog, stretch of the flexors gives this effect (Sassa, 1921). These stretches were extensively studied by Liddell and Sherrington (1924, 1925). The monosynaptic relations were worked out by Lloyd (1952). More recently, by placing an electrode tip inside a motor horn cell, Eccles and his school have studied the impact of the sensory discharge from the muscle on that cell (Eccles, 1957). The fibers from the primary endings also have many polysynaptic connections whereby the discharges activate synergic muscles or inhibit the antagonists, as well as helping to bring about adjustments in the whole posture of the animal so that the original group of muscles can work to the best advantage.

C. The Central Control of the Motor Supply to Muscle Spindles

As far as we know, the small motor fibers come from little cells lying near the large motor horn cells supplying the muscle. In a long series of experiments carried out by Granit and his coworkers (Granit, 1955), it has been shown that the activity of these gamma cells can be influenced in many ways. They are excited by impulses coming from the skin over the muscles and from neighboring structures (Eldred and Hagbarth, 1954); and by impulses from certain areas in the brain, particularly from a diffuse area in the midbrain, from the anterior lobe of the cerebellum, from the hypothalamus, and sometimes from the cortex (Granit and Kaada, 1953; Eldred *et al.*, 1953). The gamma cells have a low threshold to these stimuli and may be activated before the alpha cells, thus the spindles are alerted quickly and the increased impulses from the primary endings play on the alpha cells to lower their threshold and to potentiate them for subsequent activity. An example of this in man is the excitatory effect on the knee jerk of handling the limb beforehand and of telling the patient to clench his hands. The main brainstem activating region lies in the midbrain. Graham Brown noticed the potency of this region on postural tonus in decerebrate monkeys as long ago as 1914 (p. 162). Another center in the bulbar region depresses gamma activity in the decerebrate animal. This gives a partial explanation of some of Magoun's results (1950), in which he modified reflex activity by stimulating the midbrain and bulbar reticular formations.

The more usual type of decerebrate rigidity is dependent on intact dorsal roots and on the activation of the gamma motor system. But there are very potent alpha system pathways which are independent of muscle receptors; their activity can be revealed by tying the basilar and carotid arteries in the cat. The anterior lobe of the cerebellum and part of the pons are thereby made anemic; the gamma activity is inhibited but there is intense rigidity even if the dorsal roots are cut (Granit *et al.*, 1955). It should not be lost sight of that animals in these states of rigidity are "working tools"; the decerebrate animal with high gamma activity enables work to be carried out on stretch reflexes and many other aspects of reflex activity. In normal life, no such exaggerated stretch reflexes exist, posture and smooth movement probably depend on a nicely adjusted balance between alpha and

muscles, such as the Pacinian corpuscles and the pain endings, is sketchy. Their discharges are probably quickly adapting and they may travel partly in nerves extrinsic to the muscles to signal deep pressure and pain in the higher centers.

IV. PATHOLOGY OF MUSCLE SPINDLES

It is not proposed to describe the muscle spindles in various neuromuscular diseases, but only to make some general remarks on the examination of such spindles. It is a long standing observation that the spindles persist, apparently unchanged, in many muscular diseases; they were even looked on as pathological structures since they might stand out in pathological muscle and be missed altogether in normal muscle. Even today, such mistakes are occasionally made by pathologists whose experience does not cover much muscle histology.

Undoubtedly, the gross structure of a muscle spindle does persist for a long time after nerve injury; it not only persists but often the capsule thickens and therefore stands out more sharply in sections (Byrnes, 1926). Long after some of the extrafusal muscle fibers have ceased to function and are in a state of degeneration, the intrafusal fibers may appear intact; these muscle fibers have nerve endings belonging to both the sensory and the motor systems, and if one ending remains intact it may have the property of greatly prolonging the existence of the muscle fiber. Thus in sections of muscle stained by the standard tissue stains, there may be very little to suggest abnormality of the spindle. If, however, the specific stains for nerves and their endings are used, a very different picture may result. Tower (1932) did a series of experiments in which she cut the motor or sensory roots to muscles in the cat and, after appropriate time for degeneration, examined the muscles using the Bielschowsky technique. If the dorsal root ganglia were removed, then only the equatorial regions of the spindles were affected, the nuclei of the nuclear bags were reduced in number and volume, the cross striations of the intrafusal fibers were more clearly seen over the central regions, the capsules invested the muscle fibers more closely (but were not thickened), and the large nerve fibers degenerated. If the ventral roots were cut, then the intrafusal muscle fibers and their nerves in the polar regions of the spindles atrophied, the capsules thickened, but the equatorial regions remained intact and the spindles were very conspicuous. When the whole muscle nerve was cut, the early changes in the spindles were less obvious than in the rest

The discharges from the secondary endings in muscle spindles have been much neglected in the past, for they are not easy to identify. Hunt (1953) has stated that they are concerned with the flexion reflex and Eccles *et al.* (1957) make a similar suggestion. Further information on these endings may come from a knowledge of their presence or absence in the spindles of certain muscles, for they may be numerous in the spindles of the postural muscles and less frequent in some of the spindles in the flexors; but this is not an easy point to prove conclusively. The discharges of the secondary endings may be reduced when the motor activity to the intrafusal muscle fibers is high and the muscle tissue lying under the secondary ending is contracted. The discharge would then be greatest when the motor activity was reduced or when excessive stretch to the whole muscle overcame this contraction of the spindle muscle fibers. The function of these secondary endings might then be to give warning of overactivity and to start protective reflexes.

The tendon organs can be identified by their behavior during contraction of the whole muscle. Once their discharge starts, it travels rapidly at about 100 m. per second in large nerve fibers and then by di- or polysynaptic pathways to cause inhibition of the muscle itself and its synergists, while the motoneurons of the antagonists are activated. Eccles *et al.* (1957) say that the inhibitory actions are most commonly detected in the extensor motoneurons.

The discharges from these muscle receptors probably have effects on the gamma motoneurons; activity of the primary endings of the spindles tends to reduce the discharge in the gamma fibers, while in active contraction of the muscle, the gamma discharge is reflexly enhanced (Hunt, 1951). These effects may be due to spinal cord mechanisms, or they may be the result of the muscle receptor discharge passing to the brainstem, thalamus, and cerebellum (Mountcastle *et al.*, 1952; Laporte *et al.*, 1956), and there initiating further discharges that influence the centers concerned with gamma motor activity. The muscle receptor discharges do not go to the cerebral cortex (L. Kruger, 1956; Mountcastle, 1957), though the impulses from the joints, periosteum, skin, hairs, etc. send information from a limb to the highest centers. The muscle activity is probably largely a subconscious one, allowing of widespread reflex activity and keeping the alpha motoneurons ready for the cortical discharges which cause voluntary movement.

Our information about the functions of the other receptors near or in

muscles, such as the Pacinian corpuscles and the pain endings, is sketchy. Their discharges are probably quickly adapting and they may travel partly in nerves extrinsic to the muscles to signal deep pressure and pain in the higher centers.

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of the muscle; later the intrafusal fibers became smaller, their nuclei swelled and degenerated, the cross striations faded, the longitudinal striations became more obvious, and the fibrous connective tissue increased.

I have examined muscles from human cases of motor neuron disease and dorsal root ganglia disease and many of the muscle spindles do show the changes described by Tower. It must be remembered that many neuromuscular diseases develop slowly so that changes may be striking in one part of a muscle while another part is still normal.

In other diseases of the locomotor system, the muscles do not change histologically. There may be abnormalities or failure of the upper motor neurons. But if disturbances of posture take place, the trouble may lie in the central pathways playing on the gamma motoneurons which supply the intrafusal fibers of the muscle spindles.

V. CONCLUDING REMARKS

The receptors of a muscle are vitally important for the integration by the nervous system necessary to bring about coordinated movement. There is a fund of knowledge about the structure of these receptors and we have also learned much about their function. The three main receptors of mammalian muscles are the primary and secondary endings of the muscle spindles and the tendon endings. It is becoming increasingly certain that the first of these takes part in the stretch reflexes which are so important in posture; its discharges act directly on the motoneurons of the muscle, the discharges themselves are greatly modified by activity in the motor nerves to the intrafusal muscle fibers. Another receptor causes inhibition of the muscle when it is stimulated; this is probably the ending of the tendon organ. The third receptor has slower time relations and is therefore associated with the secondary ending of the muscle spindle and its medium sized nerve fiber. The function of this ending is not yet clear.

If we look again at the structure of the muscle spindles, there are still many problems to be solved Why are there spindles with two, one, or no secondary endings? In the spindles with no secondary endings, the muscle fibers have small motor end-plates at either pole, which bring about phasic twitch activity. In the spindles with secondary endings, there is a diffuse innervation along the shorter intrafusal muscle fibers and partly mingled with the secondary ending, as well as the motor endings at the poles. These diffuse endings may be part

of a mechanism to hold the muscle fibers in tonic contraction. There are thus both phasic and tonic intrafusal muscle fibers which may receive their motor innervation from different sources. Evidence from lower vertebrates suggests that there are both twitch and tonic contractions in intrafusal fibers, probably in separate muscle fibers. But the arrangement seen in some crustacean claw muscles where "quick" endings can start or end a contraction and "slow" endings can hold it for long periods may have some counterpart in the mammalian muscle spindle. We know that both the primary and secondary ending record degree of stretch or is the secondary ending more sensitive when the motor supply to the spindle is less active?

In whatever way the spindle works, we know that adequate stimulus to the very sensitive sensory endings causes depolarization of their membranes. Slowly adapting discharges are sent to the central nervous system; here their influence is such that by direct or indirect pathways the final common paths for muscle action are kept adjusted. We also know that the primary ending of a spindle is much more sensitive when the motor supply to the intrafusal fibers is intact, and the small gamma motor horn cells supplying the spindles are being influenced by discharges from widespread regions of the body. The motor end-plates on the intrafusal muscle fibers may have some as yet unexplored link with the motor supply of the extrafusal muscle fibers, while the diffuse endings may link up with Granit's gamma system. These diffuse endings, associated with the secondary endings, are particularly numerous in the muscles concerned with posture, and it is these muscles that seem to have many complex spindles per g. of tissue. It is probable that the explanation of muscle tone lies in the understanding of the adjustment of muscle spindles and hence of their sensory messages. The messages from many muscles combine both temporally and spatially to achieve the widespread muscular coordination which is the basis of normal movement. But there are still many problems to be investigated before we fully understand the muscle receptors.

References

Adrian, E. D. (1926). *J. Physiol.* **61**, 49.
Adrian, E. D., and Zotterman, Y. (1926). *J. Physiol.* **61**, 151.
Alexandrowicz, J. S. (1951). *Quart. J. Microscop. Sci.* **92**, 163.
Allen, W. F. (1917). *J. Comp. Neurol.* **28**, 137.
Andrew, B. L. (1955). *J. Physiol.* **130**, 474.

Asayama, C. (1916). *Quart. J. Exptl. Physiol.* **9**, 265.
Barets, A. (1952). *Arch. Anat. microscop. Morph. exp.* **41**, 305.
Barets, A. (1956). *Arch. Anat. microscop. Morph. exp.* **45**, 254.
Barker, D. (1948). *Quart. J. Microscop. Sci.* **89**, 143.
Bowden, R. E. M., and Mahran, Z. Y. (1956). *J. Anat.* **90**, 217.
Boyd, I. A. (1956). *J. Physiol.* **133**, 35P.
Boyd, I. A. (1958 a). *J. Physiol.* **140**, 14P.
Boyd, I. A. (1958 b). *J. Physiol.* **144**, 11P.
Boyd, I. A. (1959). *J. Physiol.* **145**, 55P.
Brown, G. L., and Harvey, A. M. (1938a). *J. Physiol.* **93**, 285.
Brown, G. L., and Harvey, A. M. (1938b). *J. Physiol.* **94**, 101.
Burgen, A. S. V., and Kuffler, S. W. (1957). *Nature* **180**, 1490.
Byrnes, C. M. (1926). *A. M. A. Arch. Neurol. Psychiat.* **15**, 407.
Cajal, S. R. (1888). *Rev. trim. Histol.* **1**, fasc. 1.
Cajal, S. R. (1897). *Rev. trim. Microgr.* **2**, 181.
Cardin, A. (1944). *Arch. sci. biol. (Bologna)* **30**, 9.
Cilimbaris, P. A. (1910). *Arch. mikroskop. Anat. u. Entwicklungsgesch.* **75**, 692.
Cipollone,
Coers, C.
Coers, C.,
Cooper, S. (1953). *J. Physiol.* **122**, 193.
Cooper, S. (1954). *J. Physiol.* **126**, 32 P.
Cooper, S., and Daniel, P. M. (1949). *Brain* **72**, 1.
Cooper, S., and Daniel, P. M. (1956). *J. Physiol.* **133**, 1 P.
Cooper, S., and Daniel, P. M. (1957). *Quart. J. Exptl. Physiol.* **42**, 222.
Cooper, S., and Eccles, J. C. (1930). *J. Physiol.* **69**, 377.
Cooper, S., and Fillenz, M. (1955). *J. Physiol.* **127**, 400.
Cooper, S., Daniel, P. M., and Whitteridge, D. (1951). *J. Physiol.* **113**, 463.
Cooper, S., Daniel, P. M., and Whitteridge, D. (1953). *J. Physiol.* **120**, 471.
Cooper, S., Daniel, P. M., and Whitteridge, D. (1955). *Brain* **78**, 564.
Corbin, K. B., and Harrison, F. (1940). *J. Neurophysiol.* **3**, 423.
Daniel, P. (1946). *J. Anat* **80**, 189.
Danzinger, F. (1936). *Z. Zellforsch. u. mikroskop. Anat.* **25**, 316.
Dogiel, A. S. (1902). *Arch. mikroskop. Anat. u. Entwicklungsgesch.* **59**, 1.
Eccles, J. C. (1957). "The Physiology of Nerve Cells," 270pp. Johns Hopkins Press, Baltimore, Maryland.
Eccles, J. C., and Sherrington, C. S. (1930). *Proc. Roy. Soc.* **B106**, 326.
Eccles, J. C., Eccles, R. M, and Lundberg, A. (1957). *J. Physiol.* **138**, 227.
Eldred, E., and Hagbarth, K -E. (1954). *J. Neurophysiol.* **17**, 59.
Eldred, E., Granit, R, and Merton, P. A. (1953). *J. Physiol.* **122**, 498.
Eyzaguirre, C. (1957). *J. Neurophysiol.* **20**, 523.
Eyzaguirre, C. (1958). *J. Neurophysiol.* **21**, 465
Eyzaguirre, C., and Kuffler, S W. (1955). *J. Gen. Physiol.* **39**, 87.
Fessard, A., and Sand, A. (1937). *J. Exptl. Biol.* **14**, 383.
Finlayson, L. H., and Lowenstein, O. (1958). *Proc. Roy. Soc.* **B148**, 433.
Florey, E., and Florey, E (1955). *J. Gen. Physiol.* **39**, 69.
Franqué, O. v. (1891) *Verhandl. phys.-med. Ges. Wurzburg.* **24**, 19.
Freimann, R. (1954). *Anat Anz.* **100**, 258.
Garry, R. C., and Garven, H. S. D. (1957). *J. Physiol.* **139**, 1 P.
Gerebtzoff, M A, and Grieten, J. (1956). *Compt. rend soc. biol.* **150**, 1013.
Giacomini, E. (1898). *Atti accad fisiocrit. Siena* **10**, 371, 560.
Goerttler, K. (1950). *Z. Anat. Entwicklungsgeschichte* **115**, 352.

Golgi, C. (1880). *Mem. reale accad. Torino* (2), **32**, 359.
Gordon, G., and Phillips, C. G. (1953). *Quart. J. Exptl. Physiol.* **38**, 35.
Graham Brown, T. (1914). *Proc. Roy. Soc.* **B87**, 145.
Granit, R. (1955). "Receptors and Sensory Perception," 369pp. Yale Univ. Press. New Haven, Connecticut.
Granit, R., and Kaada, B. R. (1953). *Acta Physiol. Scand.* **27**, 130.
Granit, R., Skoglund, S., and Thesleff, S. (1953). *Acta Physiol. Scand.* **28**, 134.
Granit, R., Holmgren, B., and Merton, P. A. (1955). *J. Physiol.* **130**, 213.
Granit, R., Phillips, C. G., Skoglund, S., and Steg, G. (1957). *J. Neurophysiol.* **20**, 470.
Gray, E. G. (1957). *Proc. Roy. Soc.* **B146**, 416.
Gray, J. A. B. (1959). *Progr. Biophys. and Biophys. Chem.* **9**, 285.
Hagbarth, K.-E., and Wohlfart, G. (1952). *Acta Anat.* **15**, 85.
Hebb, C., and Hill, K. J. (1955). *Nature* **175**, 597.
Hines, M., and Tower, S. S. (1928). *Bull. Johns Hopkins Hosp.* **42**, 264.
Hinsey, J. C. (1934). *Physiol. Revs.* **14**, 514.
Hoyle, G. (1957). "Comparative Physiology of the Nervous Control of Muscular Contraction." 147pp. Cambridge Univ. Press, London and New York.
Huber, G. C. (1902). *Am. J. Anat.* **1**, 520.
Hunt, C. C. (1951). *J. Physiol.* **115**, 456.
Hunt, C. C. (1952). *Federation Proc.* **11**, 75.
Hunt, C. C. (1953). *Abstr. 19th Intern. Physiol. Congr.* p. 485.
Hunt, C. C. (1954). *J. Gen. Physiol.* **38**, 117.
Hunt, C. C., and Kuffler, S. W. (1951a). *J. Physiol.* **113**, 283.
Hunt, C. C., and Kuffler, S. W. (1951b). *J. Physiol.* **113**, 298.
Jolly, W. A. (1911). *Quart. J. Exptl. Physiol.* **4**, 67.
Kadanoff, D. (1956). *Z. mikroskop. anat. Forsch.* **62**, 1.
Katz, B. (1949). *J. Exptl. Biol.* **26**, 201.
Katz, B. (1950a). *J. Physiol.* **111**, 248.
Katz, B. (1950b). *J. Physiol.* **111**, 261.
Kirsche, W. (1948). *Anat. Anz.* **96**, 419.
Kolliker, A. (1862). *Z. wiss. Zool.* **12**, 149.
Kruger, L. (1956). *Am. J. Physiol.* **186**, 474.
Kruger, P. (1952). "Tetanus und Tonus der quergestreiften Skelettmuskeln der Wirbeltiere und des Menschen," 431 pp. Geest and Portig, Leipzig.
Kuhne, W. (1863). *Arch. pathol. Anat. u. Physiol. Virchow's* **27**, 508.
Kuffler, S. W. (1953). *Arch. exptl. Pathol. Pharmakol. Naunyn-Schmiedeberg's* **220**, 116.
Kuffler, S. W. (1954). *J. Neurophysiol.* **17**, 558.
Kuffler, S. W., and Eyzaguirre, C. (1955). *J. Gen. Physiol.* **39**, 155.
Kuffler, S. W., and Hunt, C. C. (1952). *Research Publs. Assoc. Research Nervous Mental Disease* **30**, 24.
Langley, J. N. (1922). *J. Physiol.* **56**, 382.
Laporte, Y., Lundberg, A., and Oscarsson, O. (1956). *Acta Physiol. Scand.* **36**, 175.
Leksell, L. (1945). *Acta Physiol. Scand.* **10**, *Suppl.* **31**, 84pp.
Liddell, E. G. T., and Sherrington, C. S. (1924). *Proc. Roy. Soc.* **B96**, 212.
Liddell, E. G. T., and Sherrington, C. S. (1925). *Proc. Roy. Soc.* **B97**, 267.
Lloyd, D. P. C. (1952). *Research Publs. Assoc. Research Nervous Mental Disease* **30**,48.
Lucas Keene, M. F. (1957). *J. Anat.* **91**, 590.
Magoun, H. W. (1950). *Physiol. Revs.* **30**, 459.
Mather, V., and Hines, M. (1934). *Am. J. Anat.* **54**, 177.
Matthews, B. H. C. (1931a). *J. Physiol.* **71**, 64.
Matthews, B. H. C. (1931b). *J. Physiol.* **72**, 153.

Matthews, B. H. C. (1933). *J. Physiol.* **78**, 1.
Matthews, P. B. C. (1958). *J. Physiol.* **140**, 54P.
Matthews, P. B. C., and Rushworth, G. (1957). *J. Physiol.* **135**, 245.
Merrillees, C. R. (1957). *Anat. Record* **127**, 333.
Merton, P. A. (1953). *Acta Physiol. Scand.* **29**, 87.
Mountcastle, V. B. (1957). *J. Neurophysiol.* **20**, 408.
Mountcastle, V. B., Covian, M. R., and Harrison, C. R. (1952). *Research Publs. Assoc. Research Nervous Mental Disease* **30**, 339.
Onanoff, I. (1890). *Compt. rend. soc. biol.* **42**, 432.
Pascoe, J. E. (1958). *J. Physiol.* **143**, 54P.
Paulsen, K. (1958). *Z. Zellforsch. v. mikroskop. Anat.* **48**, 349.
Pezard, A., and May, R. M. (1937). *Ann. physiol. physicochim. biol.* **13**, 460.
Poloumordwinoff, D. (1899). *Compt. rend.* **128**, 845.
Regaud, C., and Favre, M. (1904). *Rev. gén. Histol.* **1**, 1.
Robertson, J. D. (1957). In Sjostrand and Rhodin. Electron Microscopy. p. 197. Almqist and Wiksell, Stockholm.
Ruffini, A. (1892a). *Atti reale accad. Lincei.* [V] *Rend.* **1**, 1°Sem., 442.
Ruffini, A. (1892b). *Atti reale accad. Lincei* [V] *Rend.* **1**, 2°Sem., 31.
Ruffini, A. (1893). *Arch. ital. biol.* **18**, 106.
Ruffini, A. (1898). *J. Physiol.* **23**, 190.
Sassa, K. (1921). *Brain* **44**, 308.
Schinkele, O. (1949). *Acta Anat.* **8**, 286.
Schulze, M. L. (1955). *Anat. Anz.* **102**, 290.
Sherrington, C. S. (1894). *J. Physiol.* **17**, 211.
Sherrington, C. S. (1897). *Proc. Roy. Soc.* **61**, 247.
Slawik, F. F. (1942). *Anat. Anz.* **93**, 133.
Slifer, E. H., and Finlayson, L. H. (1956). *Quart. J. Microscop. Sci.* **97**, 617.
Stilwell, D. L. Jr. (1957a). *Am. J. Anat.* **100**, 289.
Stilwell, D. L. Jr. (1957b). *Am. J. Anat.* **101**, 59.
Stilwell, D. L. Jr. (1957c). *Am. J. Anat.* **101**, 75.
Szentágothai, J. (1948). *J. Neurophysiol.* **11**, 445.
Tiegs, O. W. (1953). *Physiol Revs.* **33**, 90.
Tower, S. S. (1932). *Brain* **55**, 77.
Vihvelin, H. (1932). *Z. Zellforsch. u. mikroskop. Anat.* **16**, 597.
Voss, H. (1937). *Z. mikroskop anat. Forsch.* **42**, 509.
Voss, H. (1956a). *Anat. Anz.* **103**, 85.
Voss, H. (1956b). *Anat. Anz* **103**, 356.
Voss, H. (1956c). *Anat. Anz* **103**, 443.
Voss, H. (1958). *Anat. Anz* **105**, 265.
Whitteridge, D. (1955). *Quart. J. Exptl. Physiol.* **40**, 331.
Whitteridge, D (1959). *J Physiol.* **145**, 15P.
Wiersma, C A G., Furshpan, E , and Florey, E (1953). *J. Exptl. Biol.* **30**, 136.
Winckler, G. (1937) *Arch Anat Strasbourg* **23**, 219
Winckler, G., and Delaloye, B. (1957) *Acta Anat.* **29**, 114.

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CHAPTER XII

Intercalated Discs of Heart Muscle

F. S. SJÖSTRAND AND E. ANDERSSON-CEDERGREN

I. INTRODUCTION

In light microscopy, the intercalated discs of heart muscle tissue have been the subject of much controversy as to an evaluation of their existence in the living tissue, their morphology, and the interpretation of their significance from a morphological as well as a functional point of view. An extensive review of the literature up to 1945 was published in Aurell's monograph (1945). The intercalated discs were observed in 1863 by Aeby and were first described in detail by Eberth (1866), who interpreted them as consisting of a homogeneous cementing material at cell boundaries. He therefore called them "Kittstreifen".

In the earlier papers on this component, the most frequent interpretation was more or less in line with that of Eberth (Durand, 1879; Ranvier, 1889; Renaut and Landouzy, 1877; Browicz, 1889, 1890, 1893; Schiefferdecker and Kossel, 1891; Przewoski, 1893; Dunin, 1894). Wagener (1872), however, did not consider the cardiac muscle as divided into cell territories either in embryos or in adults. With the work of von Ebner (1900, 1902a, b, 1914, 1918, 1920) and of Heidenhain (1901, 1911, 1914, 1919, 1932), the syncytial continuity of the heart muscle tissue was generally accepted, and a variety of interpretations were offered regarding the significance of the intercalated discs. They were now proposed to be accidental structural modifications, for instance due to local contractions, aging, or undefined physical factors (Aschoff, 1906, 1909; Cohn, 1909; Jordan, 1911,

1912; Jordan and Steele, 1912; Jordan and Banks, 1917; von Ebner, 1918, 1920), or permanent components of importance for the arrangement of the cross-striations in connection with the branching of the heart muscle network (Heidenhain, 1901, 1911; Korner, 1933, 1935a, b, 1937; Witte, 1919), or of importance for the arrangement of the myofibrils (Marceau, 1902a, b, 1903, 1904), intramuscular tendons (Stamer, 1907; Benda, 1909; Ogata, 1914), post mortem modifications (Hoffmann, 1909; Boerner-Patzelt, 1929), or thickened parts of the ends of the myofibrils (Przewoski, 1893; Hoche, 1897; Dietrich, 1906, 1909, 1910; Bruno, 1928). The interpretation that the intercalated discs represented cell boundaries was, however, repeatedly proposed (Tawara, 1906; Szymonowicz, 1901; Zimmermann, 1910; von Palczewska, 1910; Werner, 1910; Schafer, 1910).

II. LIGHT MICROSCOPY

In an extensive study of the light microscopy of the intercalated discs, Aurell (1945) showed by means of three-dimensional reconstructions that all projections of the discs located at various levels within a limited region along a muscle fiber cover the whole cross section of the fiber. The most obvious consequence of this observation, namely that the intercalated discs had to do with transversally oriented cell boundaries, was not discussed presumably because the idea of a syncytial arrangement of the heart muscle tissue was dogmatically accepted.

The intercalated discs appear in the light microscope as transversally oriented bands which can be differentially stained. They are located at points where the muscle fibers branch, and each disc extends across only part of the cross section of the branch. Several discs are arranged in a characteristic steplike fashion. The thickness of the discs varies in different species from less than the length of a sarcomere to values exceeding that of the sarcomere length. The position of the discs was described as corresponding to that of the Z line (Jordan and Steele, 1912), or of the Z line and the I band (von Ebner, 1900, 1902a, b). They are interposed between two sarcomeres with a Z membrane covering their two broad surfaces (Heidenhain, 1901, 1911).

The detailed structure of the intercalated discs was interpreted by light microscopists as representing longitudinally oriented short rods (Wagener, 1880; Browicz, 1889, 1893; Hoche, 1897; Heidenhain, 1901, 1911) or a porous disc (Aurell, 1945).

The idea of a syncytial arrangement of the heart muscle tissue has been accepted by physiologists as explaining, for instance, the electrophysiological characteristics of the cardiac muscle and the all-or-none behavior of the heart muscle.

III. ELECTRON MICROSCOPY

The electron microscopic study of thin sections through heart muscle tissue has clearly demonstrated that the intercalated discs represent a special differentiation of the sarcoplasm in connection with transversally oriented cell boundaries (Sjöstrand and Andersson, 1954). There is no doubt that the heart muscle is subdivided into cell territories and does not represent a syncytium. The dimensions of the intercalated discs obviously show that some other components are involved in addition to the plasma membranes of the adjacent heart muscle cells. In fact, a region of the sarcoplasm located at the plasma membrane appears particularly dense and is heavily stained by means of osmium tetroxide. It is this zone of dense sarcoplasm that makes it possible to observe the intercalated discs by means of light microscopy.

The definite demonstration of cell boundaries running across the branches of the heart muscle tissue depended on a clear cut demonstration of the behavior of the plasma membrane itself at the intercalated discs. This demonstration is technically rendered difficult due to the high opacity of the sarcoplasm in these regions.

In a low resolution study by van Breeman (1953), it was concluded "that the intercalated discs are structures produced by collagenous invasion at cell wall junctions." The reason for this conclusion was that he interpreted the opaque zone of the sarcoplasm at the cell boundary as representing extracellularly located material ("the disc is not intracellular") derived *inter alia* from the collagenous components which were assumed to be associated with the sarcolemma. From this, it is obvious that van Breeman had not been able technically to surpass in any crucial way the contributions of light microscopy (compare for instance the papers mentioned above in which the intercalated discs were interpreted as intramuscular tendons).

The definite direct proof that the intercalated discs are associated with cell boundaries and that the heart muscle consists of well defined cell territories needed a high resolution study on ultrathin sections (Sjostrand and Andersson, 1954). It was then possible to visualize the

opaque osmiophilic layer of the plasma membrane at the intercalated discs and thus to observe that the plasma membrane, which covered the lateral surface of the branches of muscle tissue, changed its orientation at the intercalated disc to roughly transversal. In the sections, it was possible to follow its more or less wavy course across the muscle tissue branch. This orientation could be changed into a longitudinal course to resume the transversal orientation after some distance in accordance with the steplike appearance of the intercalated discs observed in light microscopy.

The observations made by Sjöstrand and Andersson were confirmed by Poche and Lindner (1955), Price *et al.* (1955), Lindner (1957), Moore and Ruska (1957), and by Sjöstrand *et al.* (1958).

A. The Sarcolemma

The sarcolemma appears in the electron microscope as consisting of two opaque layers separated by a more or less well defined, less opaque interspace. The one of these opaque layers, which is most adjacent to the sarcoplasm of the heart muscle cells, is sharply outlined and of a uniform thickness in each species but varies somewhat with the species of animal. Its dimension corresponds to that of the opaque layer of the plasma membrane of other types of cells.

The other opaque layer shows diffuse outlines, especially when examined in very thin sections which allow the most detailed study. The opacity of this layer then appears less pronounced than that of the other layer. It shows all the morphological characteristics of what is generally described as basement membrane in electron microscopic studies and is morphologically identical to the basement membrane of skeletal muscle cells.

This basement membrane delimits the connective tissue spaces extending through the meshes of the heart muscle network in a similar way as the basement membrane of epithelia forms a boundary between epithelium and connective tissue. The basement membrane will here be considered as a part of the sarcolemma which, in addition to this membrane, consists of the plasma membrane of the cardiac cell. The opaque layer will here be considered to represent one component of the plasma membrane which is osmiophilic. It will be referred to as the o-layer of the plasma membrane. It seems reasonable not to exclude the possibility that the plasma membrane includes an additional, less osmiophilic, light layer.

B. The Various Regions of the Intercalated Discs

The detailed structural organization of the intercalated discs shows considerable differences in various species (Figs. 1, 4, and 6). In general, several zones or regions can be distinguished along the intercalated discs showing considerable structural differences. We can in most cases differentiate the regions where the myofibrils reach near the plasma membrane at the intercalated disc from those regions where the plasma membrane of intercalated disc covers larger sarcoplasmic spaces. In the latter region, the myofibrils are separated by rows of mitochondria. Within this latter region, the cell boundary can be organized according to two different alternatives. Where the cell boundary is oriented parallel to the myofibrils and extends between two steps of the intercalated disc, still another pattern can be observed. These different regions will be referred to as the interfibrillar region, the intersarcoplasmic region, and the longitudinal connecting surfaces of the intercalated disc (Fig. 1).

The cell boundary at the intercalated disc follows a wavy course when observed in sections oriented parallel to the myofibrils. The amplitude of the waves varies considerably with the species. It is greater in mouse and guinea pig than in frog and toad cardiac muscle. The waves are especially irregular and show narrow peaks within the interfibrillar regions. In the mouse and guinea pig, the orientation of most of the cell boundary will therefore be at a rather acute angle to the orientation of the myofibrils.

It is these waves that have appeared in the light microscope as rods or pores. However, they frequently appear as a zig-zag line in, for instance, the cow cardiac muscle, even in the light microscope (Aurell, 1945).

C. The Plasma Membranes at the Intercalated Discs

The plasma membranes at the intercalated discs (Figs. 2, 5-7) consist of an opaque osmiophilic layer, the o-layer, which is continuous with that covering the lateral surfaces of the muscle cell branches (Fig. 2). The two o-layers of the two joining cells are separated by a less opaque interspace, frequently of a rather constant width within a certain region along the surface of contact. The width of this interspace, which here will be called the l space, differs in various species and in different regions along the intercalated disc. The



FIG. 1. Section through an intercalated disc of mouse cardiac muscle. IF, interfibrillar region; IL, longitudinal connection between two steps; IS, intersarcoplasmic region; M, mitochondrion; S, S-region; SA, sarcolemma. Phosphotungstic staining after osmium fixation. Magnification: × 57,000.



FIG. 2. Section through an S region and the lateral edge of an intercalated disc (mouse) where the plasma membrane changes orientation from covering the lateral surface of a cardiac muscle cell to pass into the intercalated disc area. DN, disc network; L, l space, M, mitochondrion; O, o-layer; S, S region; SA, sarcolemma; X, l space oriented obliquely to the plane of the section. Phosphotungstic staining after osmium fixation. Magnification: × 95,000.

TABLE I

THE WIDTH IN ANGSTROM UNITS OF THE I SPACE AT THE INTERCALATED DISC

Species	Inter-fibrillar region	Inter-sarcoplasmic region	Specialized region	Longitudinal connecting surfaces
Mouse	130	130	95	90
Guinea pig	70		130	50
Frog	120	120	—	—

dimensions are presented in Table I which shows that 120–130 A. is a common figure for the width.

Other figures that have been reported are 150–200 A. for the dog cardiac muscle (Poche and Lindner, 1955); 130–150 A. for the dog, rat, and mouse cardiac muscle (Moore and Ruska, 1957); and 80–200 A. for the frog cardiac muscle (Lindner, 1957).

In the mouse cardiac muscle, the o-layer of the plasma membrane appears as a triple-layered structure at the intercalated disc (Fig. 2). This triple-layered structure has been observed within limited regions of the plasma membrane. The plasma membrane is covering such an irregular surface at the intercalated disc that its orientation with respect to a longitudinal section through the disc will vary considerably. The triple-layered appearance can only become visible in such places where the o-layer is oriented exactly perpendicularly to the plane of the section. Therefore, it cannot be excluded that the o-layer along the whole intercalated disc is in fact triple-layered. Indications of a triple-layered appearance of the o-layer of the sarcolemma have also been observed on phosphotungstic acid stained material. The sublayers consist in this case of two strongly osmiophilic layers and an interposed, less osmiophilic layer. The thickness of each layer is about 25 A., and the total thickness of the triple-layered structure is 75 A.

In the guinea pig, a similar subdivision into three layers is observed within certain areas in the intersarcoplasmic regions (Fig. 5). The most peripheral opaque sublayer is sharply outlined. The other opaque sublayer appears diffusely outlined towards the cytoplasm, presumably when not oriented exactly perpendicularly to the plane of the section, due to the fact that the cytoplasm is particularly opaque inside this sublayer. The total thickness of the triple-layered opaque part of the plasma membrane is 80 A. and the thickness of the sublayers is 20 A. for the opaque layers and 40 A. for the inter-

posed light layer. A similar subdivision into three layers has been observed in the toad.

In the guinea pig, the plasma membrane at interfibrillar regions appears as a single opaque layer measuring 40 A.

The crucial proof that the intercalated discs are located at transversally oriented cell boundaries was presented by demonstrating that the o-layers of the plasma membrane of two cell territories are separated. This was done by showing that the o-layer of the intercalated disc is continuous with the o-layer of the plasma membrane covering the lateral surfaces of the muscle fibers (Sjöstrand and Andersson, 1954).

A detailed analysis of the relations at the edges of the intercalated discs clearly shows that the o-layers of the plasma membranes at the two cell territories are distinctly confined to each territory and do not fuse (Fig. 2). At the edge of the disc, no longitudinally oriented connection between the opaque layers of the two cell territories could thus be observed. This excludes the possibility that the opaque membrane components at the intercalated discs are intracellularly located membranes.

In the electron micrographs, the cell boundaries can be observed traversing the branches of the muscle tissue completely. The complete separation of the cell territories has not been established three-dimensionally in any electron microscopic study so far. This would involve an analysis of a great number of serial sections. However, if the intercalated discs were only incomplete septa, this would show up in two dimensions as well by the appearance of incomplete transversal boundaries. This has not been the case. In this connection, the careful three-dimensional reconstruction made by Aurell (1945) from light microscopic pictures is of importance. Since they showed that the various areas of intercalated discs cover the whole cross section of a muscle fiber, the reasonable conclusion is that the heart muscle branches are completely divided by the intercalated discs. With our present knowledge regarding the morphology of the intercalated discs, we are justified in concluding that the cardiac muscle is completely divided into cell territories.

At the edge of the intercalated disc, the basement membrane bridges the gap between the opaque layers of the plasma membranes without showing any deviation from its course, as was pointed out by Lindner (1957).



Fig. 3. Section through intercalated disc of mouse cardiac muscle showing the relations between the myofilaments and the disc network. A and I, A and I band of a myofibril, DN, disc network; IL, longitudinal connection between two steps; M, mitochondrion; S, S region. Phosphotungstic staining after osmium fixation. Magnification: × 75,000.

D. Cytoplasmic Differentiations at the Intercalated Discs

The different regions of the intercalated disc are characterized by the specialized organization of the cytoplasm within a narrow zone at the plasma membrane. Along most of the intercalated disc, this zone of cytoplasm is particularly dense and intensely stained with, for instance, osmium. The density varies with the species. In the frog, the density is much less pronounced than in the mouse and guinea pig. This might be the explanation for the fact that intercalated discs have been reported as missing in frog cardiac muscle. With decreasing thickness of the sections, the density becomes less apparent, because we are approaching the conditions of a two-dimensional object. This has to be considered when studying the pictures illustrating this chapter.

The structural organization of the cytoplasm is strikingly different in the interfibrillar as compared to the intersarcoplasmic regions.

In the interfibrillar regions of the intercalated discs, a network is formed by very fine filaments, which make contact with the o-layer of the plasma membrane, and extend in the mouse cardiac muscle about 0.1 μ towards the interior of the cell body (Fig. 3). The filaments, which form this dense network by an irregular crisscrossing, do not appear very well defined as to the dimension of their diameter. This is presumably due to their small dimensions and irregular arrangement.

The I band part of the myofilaments makes contact with this network and seems to be continuous with the filaments of the network. It has so far not been possible to differentiate between the myofilaments and other filamentous components of the network.

No filaments bridge the l space separating the o-layers of the plasma membrane. Therefore, there is no indication of a continuity between myofibrils of adjacent heart muscle cells.

The intercalated discs are always located at the end of a sarcomere and it is therefore the I band part of the myofilaments that mixes with the disc network.

In the intersarcoplasmic regions of the intercalated discs, we find more or less distinctly outlined stretches along the cell boundary where the cytoplasm is very dense and osmiophilic. These specially differentiated regions will here be referred to as the S regions. There are definite species differences as to the details of these structural organizations.

In the mouse, the S regions are very sharply outlined and appear



ardiac
point
otonic
buffered osmium solution Magnification: × 40.000.



FIG. 5. Higher magnification of a central area of Fig. 4. The difference between the structure of the cell boundary in the intersarcoplasmic and in the interfibrillar regions is observed. Note the several S regions (S). At X, the orientation of the cell boundary is obliquely to the plane of the section. Fixation in isotonic buffered osmium solution. Magnification· × 95,000.



Fig 4 Survey picture of section through intercalated disc of guinea pig cardiac muscle. IS, intersarcoplasmic region, M, mitochondrion; S, S region. Arrows point to the edges of the intercalated disc, IF, interfibrillar region. Fixation in isotonic buffered osmium solution Magnification: × 40,000.

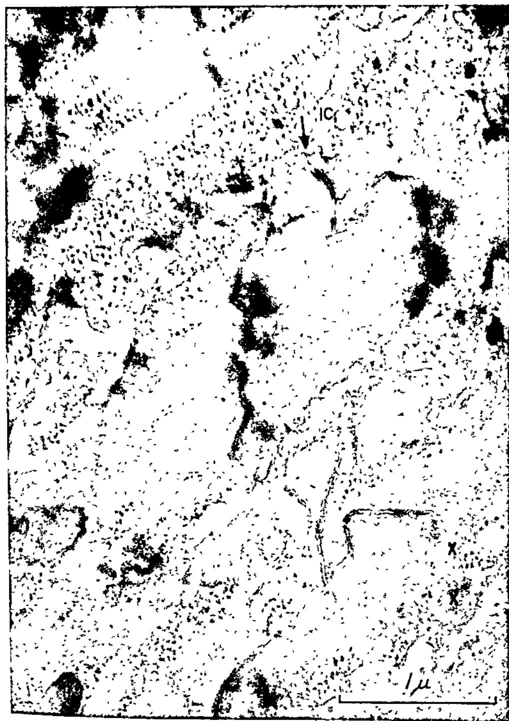


FIG. 6. Survey picture of section through intercalated disc of frog cardiac muscle. The parallel course of the o-layer of the two plasma membranes of the cell junction can be followed over long distances. The edge of the intercalated disc (indicated by an arrow) can be observed at the intercellular space (IC) in the upper part of the picture. At X, the cell boundary is oriented obliquely to the plane of the section. Magnification × 33,000.

frequently in sections cut perpendicularly to the cell boundary as opaque regions with rectangular outlines (Fig. 2). The opacity varies periodically in a direction normal to the cell surface, so that the opaque zone appears as multilayered with two approximately 100 A. broad, diffusely outlined, opaque layers. The most peripheral layer is continuous with the o-layer of the plasma membrane, more exactly defined, with its most adjacent, 25 A. thick, opaque sublayer. The two opaque layers of the cytoplasm are separated by a slightly less opaque and roughly equally thick zone. The opaque layers appear to consist of densely packed osmiophilic filaments.

The less opaque interspace, which separates the o-layers of the plasma membranes at the cell boundary, measures 95 A. in width in these regions and is distinctly more opaque than along the rest of the boundary. Very thin opaque lines extend across this space, connecting the most adjacent, 25 A. thick, osmiophilic layers of the two plasma membranes. Within those parts of the intersarcoplasmic regions which are lacking this specialized structure, the cell boundary appears as similar to any regular boundary between densely packed cells. The l space interposed between the cell territories measures here 130 A.

In the guinea pig (Figs. 4 and 5), the corresponding regions appear somewhat simpler. They correspond to those regions where the o-layer of the plasma membrane is divided into three sublayers. Only one zone of opaque cytoplasm, which gradually fades towards the interior of the cell, has been observed so far. No difference in opacity of the l spaces of the two plasma membranes could be observed. It is characteristic that, at the S regions, the cell boundary shows a rather straight or only slightly curved course in contrast to the irregularly wavy course in the interfibrillar regions. They frequently appear along longitudinally oriented surfaces of the cell boundary and seem sometimes to extend into the interfibrillar regions. Whether or not this is only due to tangential sectioning through the boundary between fibrils and sarcoplasmic regions is not clear.

At the longitudinal connecting surfaces of the cell boundary, which extends between, for instance, two adjacent sarcomeres, the two plasma membranes can be in close contact (Figs. 6 and 7). When the separation between the steps is larger, an interstitial space frequently separates the two plasma membranes. When in contact, the two plasma membranes form, in mouse cardiac muscle, a layered structure with five layers, three opaque, osmiophilic layers and two less opaque, interposed layers. The

middle opaque layer is thinner than the two others which measure 40 A. in thickness. It divides the 90 A. wide interspace between the other two opaque layers into two equal halves. *There is no opaque cytoplasm associated with this type of junction between the heart muscle cells.*

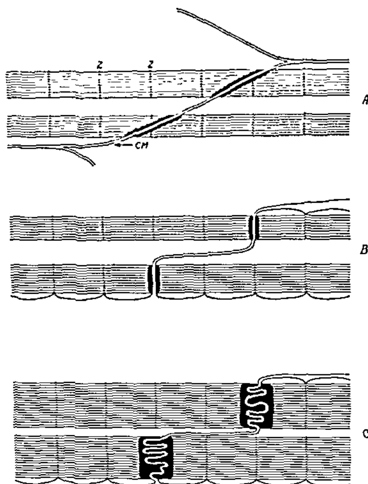


Fig. 8. Schematic drawing of three stages in the development of the intercalated disc in rabbit cardiac muscle: A, embryo; B, new born; C, adult. Redrawing from Muir (1957).

E. The Embryonic Development of the Intercalated Discs

According to light microscopic studies, the intercalated discs do not appear during the embryonic or early fetal life (Witte, 1919). They increase in numbers and complexity with age after birth (Jordan and Steele, 1912). These facts have been used as an argument against the interpretation that they represent cell boundaries.



Fig. 7. Intercalated disc of frog cardiac muscle showing the o-layers of the plasma membrane separated by the 1 space. Notice the rather obvious parallel course of the two o-layers. Magnification: × 60,000

IV. INTERPRETATION OF THE STRUCTURAL PATTERNS

There is no doubt that the intercalated discs are located at cell boundaries which separate cell territories in a more or less transversal direction in relation to the course of the myofilaments. Figure 9 gives

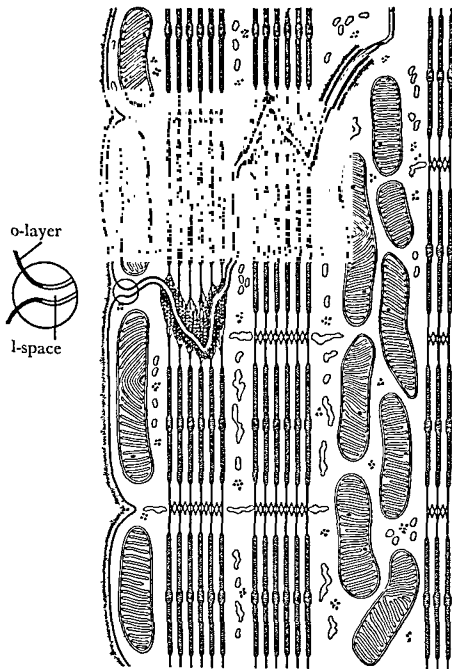


FIG. 9. Schematic drawing of intercalated disc of mouse cardiac muscle.

In an electron microscopic study of the embryonic heart muscle tissue of the rabbit, Muir (1957) has clearly demonstrated that the cardiac tissue *is divided into distinct cell territories bounded by* complete plasma membranes. In embryos, the heart muscle cells were spindle-shaped and mononucleated. Bundles of myofilaments divided into sarcomeres by Z membranes reached the plasma membrane at the end of a sarcomere. At the plasma membrane, the myofilaments were surrounded by an opaque material. In embryos from 18 days after coitus to birth, the myofibrils of two adjacent cells reached the plasma membrane at corresponding sites of the cell surfaces. The ends of the myofilaments were embedded in a material of a similar opacity as that in the intercalated discs of adult animals. The cell surface could be oriented obliquely to the axis of the myofilaments. After birth, the orientation was always perpendicular. Later on, the adult type with its characteristic wavy course of the cell boundary within the intercalated disc is developed. These stages are schematically shown in Fig. 8.

The embryonic heart muscle cells are mainly mononucleated, but the cells can contain two nuclei, according to Muir's observations made by means of phase contrast microscopy.

The human adult cardiac muscle cells have been reported to contain one to two nuclei (von Palczewska, 1910). This seems to be the most common case in a variety of animal species as reported by Werner (1910). The observations that the cells in some cases could contain 4–32 nuclei (Werner, 1910) might well be explained by incomplete staining of the cell boundaries.

The failure of light microscopists to observe the intercalated discs in embryonic heart tissue is easily explained by the minute dimensions of the embryonic discs. In early embryonic life, the discs are confined to the scattered myofibrils reaching the cell surface. With further development of the myofibrils, the discs become larger but still very thin. After birth, when the wrinkling of the cell surface at the discs is developed, the disc zone becomes easy to observe.

As the multinucleated adult cardiac muscle cell is much larger than the mononucleated cell of the embryonic muscle, the myofibrils must grow in length by adding new sarcomeres. Muir draws the attention to the fact that Heidenhain's (1901) suggestion that the intercalated discs were the site of growth of the myofibril is not incompatible with our present knowledge.

triple-layered component would represent a basic membrane component consisting of the lipids as well as of the proteins of the plasma membrane.

When trying to visualize the three-dimensional appearance of the cell boundary at the intercalated disc, it seems justifiable to assume that the wavy course of the boundary as observed in sections corresponds to

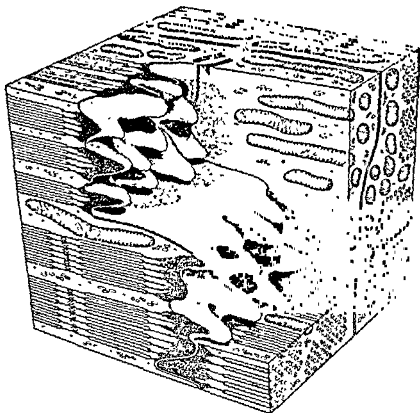


FIG. 10. Three-dimensional presentation of intercalated disc of mouse cardiac muscle. Free reconstruction based on observations made on sections, model has not been reconstructed from serial sections.

interdigitating processes as proposed by Poche and Lindner (1955) (Fig. 10). In all sections through the intercalated discs, a similar wavy course is observed, a fact that excludes the possibility that the waves are cross sections through ridges of any appreciable length. In tangential sections, the transversally cut parts of the plasma membranes form a network. This pattern is easily explained by assuming that the cell surface at the intercalated disc has the form of tightly *arranged* processes which correspond to impressions of identical form in the cell

a schematic presentation of the observations made on mouse cardiac muscle. As the myofilaments are interrrupted at the cell boundaries, there is a mechanical problem involved in connection with the development of tension in the muscle tissue. A sufficient mechanical strength has to be secured at the junction between the myofibrils and the plasma membrane on the one hand and at the contact surfaces of the two plasma membranes on the other.

The dense network that has been observed in the intercalated disc cytoplasm, the disc network, appears to secure a broad anchoring of the myofilaments to the plasma membrane. Through this network, the insert of each myofilament is broadened and the tension exerted on the plasma membrane is evenly distributed over the part of its surface that is located at the ends of the myofibrils.

The mechanical requirements at the cell boundary make it justifiable to assume that the l space separating the o-layers of the two plasma membranes is filled with some connecting material, presumably oriented lipid molecules. This assumption is supported by the fact that the width of this interspace is fairly constant along the cell boundary and is of the correct dimension to accommodate for one to two double layers of lipid molecules. It seems rather unlikely that this interspace would correspond to a water phase containing salt ions.

That the junction between the heart muscle cells is very firm is indicated by the drastic treatment that is needed for separating the cells. The old light microscopists used 35–40% KOH for this purpose. The effect of alkali could be interpreted as supporting the assumption that lipids are involved in cementing the two cell surfaces at the intercalated discs.

The width of the l space is of the same order of magnitude as that found in cell boundaries of a variety of cell types. It is proposed that the l space be considered as part of the two joining plasma membranes, and that it constitutes the lipid component of the plasma membranes.

The subdivision of the o-layer of the plasma membrane into two approximately 25-A. thick, osmiophilic layers and one interposed, less osmiophilic layer can be explained in various ways. One explanation could be that the electron stain reacts mainly with the side chains located at the two surfaces of a protein layer. A similar subdivision has been observed by Zetterqvist (1956) in the intestinal epithelium and by Robertson (1957) in the Schwann cell plasma membrane. Robertson has proposed an interpretation according to which the

of the plasma membrane in these regions correspond closely to the scheme proposed by Danielli and Davson (1934) and Danielli (1936) according to which the plasma membrane consists of a layer of lipid molecules sandwiched between two protein layers.

The S regions located at the intersarcoplasmic regions are morphologically very well defined. Their functional significance is obscure.

V. THE PROPAGATION OF EXCITATION AT THE INTERCALATED DISCS

The assumption that the heart represented a syncytium has hitherto helped in understanding the functional properties of heart muscle tissue. Physiological analyses have revealed that the propagation of the stimulus through the heart muscle tissue takes place without any appreciable synaptic delay. How is such a property compatible with a division of the heart tissue into numerous cell territories?

A possibility is that conduction from cell to cell would be based on the action of local currents. The cell structures at the surface of contact, being of a structure of similar appearance as the rest of the cell surface, would most likely form a high electrical resistance in the current path, thereby limiting the current spread and causing a low safety factor for conduction across the cell boundaries. An additional attenuation of the local excitatory currents would be brought about if the l space would consist of a water phase containing salt ions. A somewhat remote speculation is that the S regions would have a lower resistance, thereby forming paths with an increased safety factor for conduction across the cell boundaries.

The intercalated discs represent an example where the interpretation that the well defined, less opaque interspaces, which are so frequently observed in connection with various membranes, represent the lipid components of the membranes (Sjostrand, 1953), is of good help in understanding the functional implications.

The high concentration of certain enzymes at the intercalated discs made Bourne (1953) propose that they might act as boosters of the contraction wave spreading through the cardiac muscle tissue at the heart beat.

REFERENCES

Aeby, C. (1863). *Z. ration Med.* **17**, 195.
Aschoff, L. (1906). *Verhandl. deut. Ges. Pathol.* **10**, 45.
Aschoff, L. (1909). *Verhandl. deut. Ges Pathol.* **13**, 196.

surface with which it is in contact. Through the irregular form of the cell boundaries, the surface area of contact is increased. It seems justifiable to assume that, with increasing area, the mechanical stability of the junction is improved.

The surface area, on which the pull of each myofilament is transferred, is increased both by the network of the cytoplasm at the interfibrillar regions and by the increase of the plasma membrane surface by its wrinkled arrangement. The waviness of the plasma membrane also means that the direction of the pull will be more or less oblique relative to the plane of the plasma membrane.

The wrinkling of the plasma membrane at the intercalated disc certainly means that an adjustment to the variations of the diameter of the muscle cell branches at different states of activity.

The great similarity of the appearance of the intercalated discs to that of certain other specialized regions of intercellular contact was pointed out by Sjostrand and Andersson (1954). The epidermal cells of the frog skin (Ottoson *et al.*, 1953), the terminal bars of, for instance, the exocrine pancreas cells (Sjöstrand and Hanzon, 1954), and the so-called external limiting membrane of the retina of, for instance, the guinea pig eye (Sjostrand, 1953) were the examples that were mentioned. It can be added that the connections between smooth muscle cells are rather similar (Bergman, unpublished). Recently, Horstmann and Knoop (1958) have demonstrated a structural organization of the Bizzozero's nodules in the rat pad skin which appears rather similar to the S region of the intercalated discs. For the external limiting membrane of the retina (Sjöstrand, 1953), it was proposed that the dense region of the cytoplasm represented a kind of intracellular skeleton at the site of specially firm connections with the surrounding cells. It seems justifiable to assume for the cardiac muscle, as was done for the external limiting membrane, that the dense cytoplasm plays more than a purely mechanical role. The high enzyme concentration in this region (Bourne, 1953) thus indicates a high metabolic rate.

In the mouse cardiac muscle, the cell boundary at longitudinal connecting surfaces consisted of an intermediate opaque layer located between the main opaque layers of the plasma membranes. This arrangement is similar to the identity period of the nerve myelin sheath. It might be that the intermediate layer corresponds to an interposed protein layer as has been proposed for the opaque layers of the plasma membrane. Such an assumption would make the picture

Schiefferdecker, P., and Kossel, A. (1891). "Gewebelehre mit besonderer Berucksichtigung des menschlichen Korpers." H. Bruhn, Braunschweig.
Schäfer, E. A. (1910). "The Essentials of Histology," 8th ed. Lea & Febiger, Philadelphia and New York.
Sjöstrand, F. S. (1953). *J. Cellular Comp. Physiol.* **42**, 45.
Sjostrand, F. S., and Andersson, E. (1954). *Experientia* **10**, 369.
Sjostrand, F. S., Andersson-Cedergren, E., and Dewey, M. (1958). *J. Ultrastructure Research* **1**, 271.
Sjostrand, F. S., and Hanzon, V. (1954). *Exptl. Cell Research* **7**, 393.
Stamer, A. (1907). *Beitr. pathol. Anat. u. allgem. Pathol.* **42**, 310.
Szymonowicz, L. (1901). "Lehrbuch der Histologie und der mikroskopischen Anatomie." Stuber, Würzburg.
Tawara, S. (1906). "Das Reizleitungssystem des Saugetierherzens." Fischer, Jena.
van Breeman, V. L. (1953). *Anat. Record* **117**, 49.
von Ebner, V. (1900). *Sitzber. Akad. Wiss. Wien Math. naturwiss. Kl. Abt. III* **109**, 700.
von Ebner, V. (1902a). *Verhandl. morphol. physiol. Ges. Wien, see Centr. Physiol.* **16**, 566.
von Ebner, V. (1902b). "Kollikers Handbuch der Gewebelehre des Menschen," Vol. III. Engelmann, Leipzig.
von Ebner, V. (1914). *Anat. Anz.*, Erg. H, **46**, 2.
von Ebner, V. (1918). *Sitzber. Akad. Wiss. Wien Math. naturwiss. Kl. Abt. III* **127**, 3.
von Ebner, V. (1920). *Sitzber. Akad. Wiss. Wien Math. naturwiss. Kl. Abt. III* **129**, 3.
von Palczewska, I. (1910). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **75**, 41.
Wagener, G. R. (1872). *Sitzber. Ges. Beförder. ges. Naturw. Marburg* **7**, 141.
Wagener, G. R. (1880). *Arch. Anat. Entwicklungsgesch.* **4**, 253.
Werner, M. (1910). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **75**, 101.
Witte, L. (1919). *Am. J. Anat.* **25**, 333.
Zetterqvist, H. (1956). Thesis, Stockholm.
Zimmermann, K. W. (1910). *Arch. mikroskop. Anat. u. Entwicklungsmech.* **75**, 40.

Aurell, G. (1945). M. D. Thesis, Almqvist & Wiksells, Uppsala, Sweden.
Benda, C. (1909). *Verhandl. deut. Ges. Pathol.* **13**, 197.
Boerner-Patzelt, D. (1929). *Z. mikroskop. anat. Forsch.* **18**, 93.
Bourne, G. H. (1953). *Nature* **172**, 588.
Browicz, F. (1889). *Wien. klin. Wochschr.* **2**, 957.
Browicz, F. (1890). *Verhandl. 10. intern. Med. Kongr. Berlin.* [3], **2**, 87.
Browicz, F. (1893). *Arch. pathol. Anat. u. Physiol. Virchow's* **134**, 1.
Bruno, G. (1928). *Arch. ital. anat. e embriol.* **25**, 320.
Cohn, A. E. (1909). *Verhandl. deut. Ges. Pathol.* **13**, 182.
Danielli, J. F. (1936). *J. Cellular Comp. Physiol.* **7**, 393.
Danielli, J. F., and Davson, H. (1934). *J. Cellular Comp. Physiol.* **5**, 495.
Dietrich, A. (1906). *Verhandl. deut. Ges. Pathol.* **10**, 40.
Dietrich, A. (1909). *Verhandl. deut. Ges. Pathol.* **13**, 196.
Dietrich, A. (1910). *Samml. anat. physiol. Vortr.*, Heft **12**.
Dunin, T. (1894). *Beitr. pathol. Anat. u. allgem. Pathol.* **16**, 134.
Durand, A. (1879). Thesis, Lyon.
Eberth, C. J. (1866). *Arch. pathol. Anat. u. Physiol. Virchow's* **37**, 100.
Heidenhain, M. (1901). *Anat. Anz.* **20**, 33.
Heidenhain, M. (1911). "Bardelebens Handbuch der Anatomie des Menschen," Vol. 8, No. 2, p. 530. Fischer, Jena.
Heidenhain, M. (1914). *Arch. mikroskop. Anat. u. Entwicklungsmech.* [1], **85**, 365.
Heidenhain, M. (1919). *Anat. Hefte* **56**, 321.
Heidenhain, M. (1932). "Die Spaltungsgesetze der Blätter." Jena.
Hoche, C. L. (1897). *Bibliogr. anat.* **5**, 159.
Hoffmann, P. A. (1909). Thesis, Leipzig.
Horstmann, E., and Knoop, A. (1958). *Z. Zellforsch. u. mikroskop. Anat.* **47**, 348.
Jordan, H. E. (1911). *Anat. Record* **5**, 517.
Jordan, H. E. (1912). *Anat. Record* **6**, 357.
Jordan, H. E., and Banks, J. B. (1917). *Am. J. Anat.* **22**, 285.
Jordan, H. E., and Steele, K. B. (1912). *Am. J. Anat.* **13**, 151.
Korner, F. (1933). *Z. mikroskop. anat. Forsch.* **34**, 249.
Korner, F. (1935a) *Z mikroskop anat. Forsch.* **37**, 407.
Korner, F. (1935b). *Z. mikroskop. anat. Forsch.* **38**, 441.
Korner, F. (1937). *Arch. Kreislaufforsch.* **1**, 358.
Lindner, E. (1957). *Z. Zellforsch. u. mikroskop. Anat.* **45**, 702.
Marceau, F. (1902a). Thesis, Nancy.
Marceau, F (1902b). *Compt. rend. soc. biol.* **54**, 714.
Marceau, F (1903). *Compt. rend.* **136**, 1685.
Marceau, F. (1904). *Ann. sci. nat. Zool.* [8], **19**, 191.
Moore, D H , and Ruska, H. (1957). *J. Biophys. Biochem. Cytol.* **3**, 261.
Muir, A. R. (1957). *J. Biophys. Biochem. Cytol.* **3**, 193.
Ogata, T. (1914). *Frankfurt. Z. Pathol.* **15**, 127.
Ottoson, D., Sjostrand, F., Stenstrom, S , and Svaetichin, G. (1953). *Acta Physiol. Scand* **29**, *Suppl.* **106**, 611
Poche, R., and Lindner, E (1955). *Z. Zellforsch. u. mikroskop. Anat.* **43**, 104.
Price, K C , Weiss, J. M., Daikichi, H., and Smith, J. R. (1955). *J. Exptl. Med.* **101**, 687.
Przewoski, E (1893) *Arch. sci. biol St. Petersb.* **2**, 286.
Ranvier, L (1889). "Traité technique d'histologie " F. Savy, Paris.
Renaut, J., and Landouzy, M. (1877). Communication by Renaut, *Compt. rend. soc. biol.* **29**, 333.
Robertson, J. D. (1957). *J. Biophys Biochem. Cytol.* **3**, 1043.

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